# Neonatal Respiratory Control

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# NEONATAL RESPIRATORY CONTROL

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**Thesis submitted for PhD examination**

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## Declaration

I, Thomas Rossor, confirm that the work presented in this Thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the Thesis.

# Abstract

**Background**

Disruption of the development of a stable and responsive system of respiratory control may be central to neonatal apnoea and Sudden Infant Death Syndrome.

**Aims**

To test the hypotheses that sleeping position, maternal smoking and substance misuse will alter the ventilatory responses to hypercarbia and hypoxia in term infants; prematurely born infants with a lower ventilatory response to hypercarbia are at greater risk of developing apnoea, caffeine will increase this response; management of gastro-oesophageal reflux varies between NICUs, investigations that detect non-acid reflux will be more sensitive in diagnosing GORD, apnoea frequency will be greater following reflux events than before.

**Methods**

The hypoxic and hypercarbic ventilatory responses were measured in term infants. The ventilatory response to hypercarbia was measured in preterm infants soon after birth and weekly until discharge. A survey was sent to UK NICUs. Infants on the NICU were investigated with pH/MII and polysomnography. Results of Upper gastro-intestinal contrast studies were compared with the results of pH/MII study.

**Results**

Maternal substance misuse alters breathing characteristics and response to hypoxia in newborns. In these infants prone compared to supine sleeping is associated with a lower minute volume. In prematurely born infants, a lower ventilatory response to hypercarbia predicted those that would develop apnoea. Caffeine was associated with an increased ventilatory response to hypercarbia. Investigation and management of gastro-oesophageal reflux in NICUs varies widely. pH/MII increases the detection of reflux events compared to pH alone. The results of pH/MII and upper gastro-intestinal contrast study correlate poorly. Apnoea frequency is no greater following reflux than preceding, or during reflux free periods.

**Conclusion**

Risks factors for SIDS alter respiratory control; apnoea of prematurity is associated with a reduced response to hypercarbia, which is increased by caffeine; there is little evidence for a role of gastro-oesophageal reflux in the pathogenesis of apnoea.

## Published abstracts arising from the thesis

ANDRADI, G., ROSSOR, T., BHAT, R. & GREENOUGH, A. 2016. A survey of the investigation and management of gastro-oesophageal reflux disease on neonatal intensive care units in Britain. *European Respiratory Journal,* 48.

ROSSOR, T., ALI, K., TRENEAR, R., HANNAM, S., RAFFERTY, G. & GREENOUGH, A. 2014a. The effects of sleeping position on the ventilatory response to hypercarbia. *European Respiratory Journal,* 44.

ROSSOR, T., ALI, K., TRENEAR, R., HANNAM, S., RAFFERTY, G. F. & GREENOUGH, A. 2014b. G113(P) The effects of sleeping position and maternal smoking on the ventilatory response to hypoxia. *Archives of Disease in Childhood,* 99**,** A48.

ROSSOR, T., ALI, K., TRENEAR, R., HANNAM, S., RAFFERTY, G. F. & GREENOUGH, A. 2015a. G114(P) The effects of sleeping position on the ventilatory response to hypoxia and hypercarbia. *Archives of Disease in Childhood,* 100**,** A50.

ROSSOR, T., BHAT, R. & GREENOUGH, A. 2015b. Evaluation of Upper Gastro-intestinal contrast studies in the diagnosis of gastro-oesophageal reflux disease in infants. *World Association of Perinatal Medicine.* Madrid.

ROSSOR, T., LINGAM, I., BHAT, R. & GREENOUGH, A. 2015c. Acid and non acid gastro-oesophageal reflux and apnoea in infants. *European Respiratory Journal,* 46.

ROSSOR, T., LINGAM, I., BHAT, R. & GREENOUGH, A. 2015d. Evaluation of combined multichannel intraluminal impedance and pH studies on the NICU. *World Association of Perinatal Medicine.* Madrid.

## Publications arising from the thesis

ROSSOR, T., ANDRADI, G., ALI, K., BHAT, R. & GREENOUGH, A. 2017a. Gastro-Oesophageal Reflux and Apnoea: Is There a Temporal Relationship? *Neonatology,* 113**,** 206-211.

ROSSOR, T., ANDRADI, G., BHAT, R. & GREENOUGH, A. 2017b. Investigation and management of gastro-oesophageal reflux in United Kingdom neonatal intensive care units. *Acta Paediatr*.

# Acknowledgements

I have thoroughly enjoyed my time working on the Fred Still Neonatal unit at King's College Hospital. My heartfelt thanks must go to Professor Anne Greenough for her tireless support, guidance and patience. My thanks to Dr Ged Rafferty for his good humour, enthusiasm and again patience, and for somehow finding a way to keep the equipment functioning despite my best efforts. My thanks also go to Professor Milner for all the gentle nudges in the right direction without which I would have got thoroughly lost.

My gratitude to Dr Kamal Ali and Dr Ravi Bhat for sharing their knowledge, wisdom and experience in the art of newborn measurements, and all the staff of the Neonatal Unit for making me so welcome during my time there. My particular thanks to the participants of the studies, and their families who made the work possible, and the nursing and midwifery staff for their help and understanding, and in particular the help of Anne Sayers.

My warmest thanks to Deirdre Gibbons for her kindness and enormous support throughout my studies, and in particular with the preparation of this thesis. I would also like to thank my research colleagues Prashanth, Sanja, Rupa, Claire, Sandeep, Alan and Vicky for their good humour and tolerance over the years.

And thank you to Tina, for her patience and almost uncomplaining support over the last five years.

# Abbreviations

| Abbreviation | Meaning |
|---|---|
| 5-HT | 5-Hydroxy-triptamine (Serotonin) |
| ACT | Acid clearance time |
| ADP | Adenosine-di-phosphate |
| AMP | Adenosine-mono-phosphate |
| AOP | Apnoea of prematurity |
| ATP | Adenosine-tri-phosphate |
| AUROC | Area under receiver operator characteristic |
| BAEP | Brainstem auditory evoked potentials |
| BCT | Bolus clearance time |
| BPD | Bronchopulmonary dysplasia |
| BTS | Back to sleep |
| CAP | Caffeine for apnoea of prematurity (trial) |
| CB | Carotid bodies |
| ccRTN | Chemically characterised Retro-trapezoid nucleus |
| CEDIA | Cloned Enzyme Donor ImmunoAssay |
| CI | Confidence interval |
| $CO_2$ | Carbon dioxide |
| E.Coli | Escherichia coli |
| FRC | Functional residual capacity |
| GABA | γ-amino butyric acid |
| GOR | Gastro-oesophageal reflux |
| GORD | Gastro-oesophageal reflux disease |
| ISAM | Infants of substance abusing mothers |
| LC | Locus coeruleus |
| LCR | Laryngeal chemoreflex |
| LPS | Lipopolysaccharide |
| MIF | Mean inspiratory flow |
| MII | Multichannel intraluminal impedance |
| MR | Medullary raphe |
| NICU | Neonatal intensive care unit |
| NMDA | N-Methyl-D-Aspartate |
| NREM | Non-rapid eye movement |
| NTS | Nucleus tractus solitarius |
| OR | Odds ratio |
| PBC | Pre-Botzinger complex |
| PCA | Post-conceptual age |
| $pCO_2$ | Partial pressure of Carbon dioxide |
| PDA | Patent ductus arteriosus |
| PDGF | Platelet derived growth factor |
| pH/MII | Combined pH and multichannel intraluminal impedance study |
| Phox2b | Paired homeobox 2b |
| PMA | Post-menstrual age |
| $pO_2$ | Partial pressure of Oxygen |
| REM | Rapid eye movement |
| RI | Reflux index |
| ROC | Receiver operatory characteristic |
| RTN | Retro-trapezioid nucleus |
| SIDS | Sudden Infant Death Syndrome |
| SNP | Single-nucleotide polymorphism |

| | |
|---|---|
| UGI | Upper gastro-intestinal contrast study |

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# Chapter 1 : Introduction

## 1.1 Historical perspective

Since mediaeval times 'overlaying' was seen as a principal means of infanticide, yet with recognition that death by overlaying was often accidental it was met with a degree of leniency.(Russell-Jones, 1985) As early as 700 AD this crime was seen as warranting a lesser punishment than the accidental killing of an adult; the former warranting three years of penance (one on bread and water) while the latter required five years (three on bread and water).(Savitt, 1979) Fourteenth century instructions to English parish priests make reference to the venial (pardonable) sin of overlaying one's child. In renaissance Florence, concern over accidental overlaying of the infant led to the production of the 'arcuccio' (little arch). This wooden frame was placed over the infant to prevent the mother rolling onto the infant, or bedding smothering them. These gained popularity and were widely used into the 19$^{th}$ century. Dr Arnold Paltauf presented data in 1889 showing that 59% of unexplained deaths in infants were occurring between two and four months of age.(Paltauf, 1889)

The police surgeon reports from Dundee in 1892 stated that 60% of the cases of overlaying in a ten year period occurred between the ages of two and four months. In presenting those data the police surgeon stated: 'the principal causes producing this great mortality from overlaying are – i) Ignorance and carelessness of mothers; ii) drunkenness; iii) overcrowding.(Templeman, 1892)

It was not until the second half of the twentieth century that the first theories of other causes of sudden death in infancy were proposed. In 1960 Parish and Barrett suggested hypersensitivity to aspirated milk in the pathogenesis of sudden infant death.(Parish et al., 1960) In 1965 an 'Enquiry into sudden death in infancy' was established in England and research priorities agreed.(Russell-Jones, 1985) Subsequently numerous theories have been proposed to explain SIDS. The apnoea theory, first proposed in 1972 by Steinschneider (Steinschneider, 1972) suggested failure of respiratory control during sleep as a critical factor in the pathogenesis of SIDS.

“Apnoea”, a Latin modernisation of the Greek apnoia meaning an inability to breathe was recognised in infants since the eighteenth century. In 1799 in ‘A Treatise on the Diseases of Children’ Dr Underwood wrote of “an infant born at the full term lay moaning and languid for four to five hours and was then seized with a fainting fit...it had ceased to breathe except now and then” in what was possibly the first description of neonatal apnoea.(Underwood, 1799) In 1923 George Frederic Still described a series of five cases of probable apnoea in a paper titled ‘Attacks of arrested respiration in the newborn’(Still, 1923) noting that these episodes of arrest occurred without warning in otherwise healthy infants and concluding that they were more likely to result from impairment of the respiratory centre than a primary lung pathology. Quantitative studies of respiration in preterm infants were carried out first by Shaw and Hopkins(Shaw LA, 1931) using a trunk plethysmograph and later by Wilson et al.(Wilson JL, 1942) Both studies remarked on the variability of rate and depth of breathing, while Wilson et al. noted a periodicity to the pattern of respiration. Howard and Bauer confirmed these findings in 1949,(Howard PJ, 1949) and that the periodic respiration was associated with apnoea. Furthermore, they observed that these patterns while more common in prematurely born infants were present in most infants when hypoxic.

In 1974 Johnson presented a paper entitled ‘Laryngeal induced apnea’ at a symposium on sudden and unexpected deaths in infancy, proposing a role for gastro-oesophageal reflux (GOR) in the pathogenesis of prolonged apnoea, potentially precipitating SIDS.(Johnson, 1974) This theory was further explored by Herbst et al. using pH probes to evaluate a possible link between GOR and recurrent apnoea in fifteen infants, concluding that GOR had a causative role.(Herbst et al., 1979) In several centres, investigation for GOR was included in the evaluation of infants considered at high risk of SIDS These studies provided the reference ranges used to interpret infant pH studies to this day.(Vandenplas et al., 1991) The role of GOR in the pathogenesis of apnoea however remains speculative, as there have been inconsistent results from numerous studies.

## 1.2 Respiratory control

Control of breathing is complex and is modulated by many afferents. Ventilation plays a crucial role in maintaining homeostasis. Carbon dioxide levels significantly impact on intra and extracellular pH and therefore must be closely controlled to permit the effective action of intracellular mechanisms. Oxygen levels must be controlled to allow cellular respiration and the utilisation of energy. An effective homeostatic system allows the organism to respond to differing environmental and metabolic demands.

Control of breathing must also accommodate other processes including swallowing and vocalisation.

The primary drive, however, for breathing is largely attributed to detection of hypoxia and hypercarbia. Chemodetection occurs at different anatomical sites. Peripheral chemoreceptors are sited outside the central nervous system, primarily at the carotid bodies. Due to the high flow of blood passing the carotid bodies they are effective in rapidly detecting changes in oxygen and carbon dioxide levels in the blood.

Central chemoreceptors are primarily located in the brainstem. These receptors are thought to respond primarily to changes in pH in the extracellular fluid within the blood brain barrier, and as such may respond to changes in carbon dioxide levels more slowly than peripheral chemoreceptors.

### 1.2.1 Central chemoreceptors

It is thought that central chemoreceptors have a primary role in mediating the hypercapnic ventilatory response and numerous sites within the brainstem have been postulated to contribute. Loeschcke identified the region on the ventral surface of the medulla as chemosensitive, and primarily responsive to extra-cellular pH.(Loeschcke, 1982) The retrotrapezoid nucleus in the ventrolateral medulla, with projections to the dorsal and ventral respiratory groups, contains neurons that are intrinsically pH sensitive.(Wong-Riley et al., 2013) Furthermore, a population of neurons in this area known as chemically characterised Retrotrapezoid nucleus neurons (ccRTN) have been characterised as expressing Phox2b

(Paired homeobox 2b) and having glutamatergic activity. The extension of these neurons to all the pontomedullary regions, implicated in respiratory rhythmogenesis and pattern generation and the failure of chemosensitivity in individuals with PHOX2B mutations, supports the hypothesis that they are significant contributors to the hypercapnic response.

Exposure to hypercarbia results in increased expression of cFos, a transcription factor which is rapidly and transiently induced by neuronal activation, in the RTN region, demonstrating increased activity.(Sato et al., 1992) Focal acidification of this region increases respiration in rats.(Li and Nattie, 1997) Photoactivation of ccRTN neurons transfected with channelrodopsin2 (a light-gated ion channel) increases phrenic nerve output.(Underwood, 1799) These studies support the theory that ccRTN neurons are activated by increased $CO_2$ and generate increased respiratory output. An alternative mechanism for central chemosensitivity has been postulated by Spyer et al. suggesting that a crucial role may be played by a subset of glial cells. Those glial cells are intrinsically chemosensitive, as pH induced depolarization resulted in release of ATP and activation of ccRTN neurons via a paracrine process.(Spyer et al., 2004) Blocking the purinergic P2 receptor, which binds ATP, however, had no effect on ccRTN neurons in vitro(Onimaru et al., 2012) and Guyenet and Mulkey suggested that the glial cells may augment the inherent chemosensitivity of ccRTN neurons.(Guyenet and Mulkey, 2010) It is therefore possible that while glial cells may not be essential to chemosensitivity, they may have a role in amplifying changes in pH, and damage to glial cells may inhibit this process.

Exposure of rats to 20% $CO_2$ resulted in increased expression of cFos in a large population of serotonergic neurons in the ventrolateral periaqueductal grey and ventrolateral part of the dorsal raphe nucleus. While such results do not indicate intrinsic chemosensitivity in those regions, it suggests a role for serotonergic neurons in the $CO_2$ chemoreflex.(Johnson et al., 2005) Furthermore, transgenic mice without serotonergic neurons had normal baseline ventilation and hypoxic ventilatory response, but an impaired response to inhaled $CO_2$.(Hodges and Richerson, 2008)

The chemoresponse to $CO_2$ involves numerous regions including the nucleus tractus solitarius (NTS), the medullary raphe (MR), the arcuate nucleus, the pre-Botzinger complex and the locus coereleus. Different regions appear to have a specific state dependent response to hypercarbia. Nattie & Li undertook a series of experiments focally acidifying regions of the rat brain associated with central chemoreception while the animals were awake or asleep. They determined that responses within the RTN, caudal NTS, and caudal medullary surface region occurred during wakefulness, whereas the responses in the MR and caudal NTS were in sleep.(Nattie and Li, 2010) Extensive interactions between the groups modulate a complex chemosensitive system. (Figure 1)

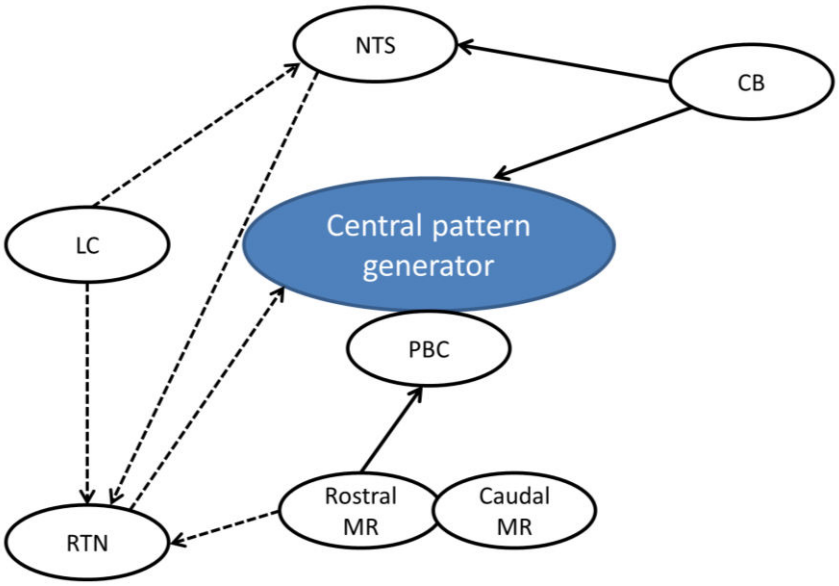


Figure 1: Schematic model for central chemoreception during nrem sleep. Solid lines represent established connections. Dashed lines represent likely connections. Retrotrapezioid nucleus (RTN); locus coeruleus (LC); medullary raphe (MR); nucleus tractus solitarius (NTS); pre-botzinger complex (PBC) and carotid bodies (CB). (Nattie and Li, 2010)

### 1.2.2 Response to hypercapnia

Increased $PaCO_2$ has been shown to increase fetal breathing movements in the lamb,(Darnall, 2010) which is unaffected by carotid body denervation suggesting the mechanism is central.(Koos et al., 1987)

Increased inspired $CO_2$ results in an increase in ventilation in both term and prematurely born infants. Sensitivity to $CO_2$ in the infant has been shown to increase with maturation.(Rigatto et al., 1975) The sensitivity of term infants to inspired $CO_2$ is reported to be similar to that of adults, but with a left shift. The baseline $CO_2$ level is lower in the infant, but the increase in minute ventilation in response to $CO_2$ is proportional to that in adults.(Avery et al., 1963) (Figure 2)

### 1.2.3 Peripheral chemoreceptors

Peripheral chemoreceptors have been localised primarily to the carotid body, with a less significant role played by chemoreceptors in the aortic body.(Loeschcke, 1982) The peripheral chemoreceptors in the carotid body are the primary site at which a response to hypoxia is initiated, but they are also responsive to hypercapnea and acidosis. The anatomical location affords rapid sensing of variations in arterial $pO_2$ and $pCO_2$ as a result of the substantial arterial blood flow through the carotid body facilitating prompt and fine control of arterial oxygenation.

There is increasing evidence, from patch clamping experiments in rat neurons, that the hypoxic response is mediated by the glomus cells in the carotid body. Gonzalez et al. suggested that an oxygen sensitive hemoprotein either forms or transduces to a K+ channel and has reduced permeability with increasing hypoxia. Loss of K+ channel activity ultimately resulted in calcium dependent depolarisation that results in neurotransmitter release.(Gonzalez et al., 2010) 5'-adenosine-triphosphate, acetylcholine and dopamine have been implicated as potential excitatory neurotransmitters. The glomus cells synapse with afferent fibres of the glossopharyngeal nerve and project to diverse areas of the brainstem, including the nucleus tractus solitarius and ventrolateral medulla.(Finley and Katz, 1992)

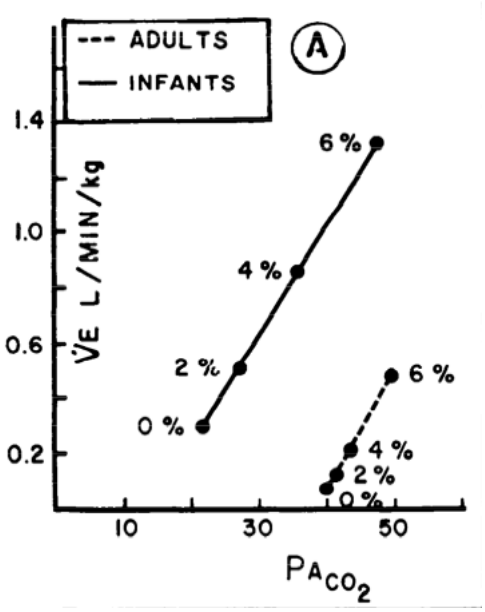


Figure 2: The ventilatory response to inspired $CO_2$ in adults and infants. The increase in minute ventilation with increasing inspired carbon dioxide is similar in both adults and infants, with the infant response shifted to the left. (Avery et al., 1963)

There is also an argument for adenosine as a mediator of hypoxic responses. With increased hypoxia, there is reduced oxidative phosphorylation of cellular AMP and therefore an increased ratio of AMP/ATP. Conversion of AMP to adenosine then results in the activation of various adenosine receptors. Adenosine receptors have been subtyped as $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$ receptors. [15] $A_1$ and $A_3$ receptors are thought to inhibit adenylyl cyclase, which converts ATP to cyclic AMP, while adenylyl cyclase is stimulated by $A_{2A}$ and $A_{2B}$ receptors.(Koos, 2011) Activation of different receptor subtypes may account for the different hypoxic responses seen in the fetus, preterm and term infant. The argument that this metabolic pathway allows $O_2$ transduction in the carotid body is supported by studies showing that oligomycin (an ATPase inhibitor), adenosine and adenosine $A_{2A}$ receptor agonists all trigger the increased respiratory rate and tidal volume seen in the hypoxic ventilatory response and this is terminated by carotid body denervation.(Koos and Chau, 1998, Koos et al., 1992)

While adenosine has an excitatory effect on ventilation when administered to the carotid bodies,(Koos et al., 1992) central administration of $A_1$ receptor agonists resulted in respiratory depression during normoxia which was blocked by non-specific adenosine receptor antagonists administration.(Bissonnette, 2000) Infusion of a specific $A_1$ receptor antagonist, however, did not alter the response to hypoxia in newborn lambs. Infusion of $A_{2A}$ receptor antagonist reduced the hypoxic ventilatory decline.(Koos et al., 2005) The role of adenosine in the response to hypoxia was further explored by administration of dipyridamole, an adenosine uptake blocker, to nine adult humans. This resulted in an increased sensitivity to hypoxia, as shown by a steeper gradient of minute volume against progressive hypoxia and subsequently an increased magnitude of hypoxic decline. Adenosine may therefore have an effect on several components of ventilatory responses. Depending on the stage of development blockade of adenosine receptors may potentially be protective or damaging. A more developed understanding of the physiological effect of adenosine receptor blockade would permit a better understanding of possible benefit or harm of therapy.

### 1.2.4 Response to hypoxia

The hypoxic ventilatory response in the newborn is characterised by a biphasic response with an initial increase or hypoxic sensory response followed by a reduction in ventilation - hypoxic ventilatory depression (Figure 3). In term infants the biphasic nature of the response develops into a mature sustained hyperpnoea, but this may not occur until at least two months of age.(Cohen et al., 1997) In contrast the fetal response to hypoxia consists of a reduction in fetal breathing movements to conserve energy. The hypoxic response in preterm infants is characterised by a slight or absent initial hypoxic sensory response, followed by a more marked hypoxic ventilatory decline similar to the fetal response.(Alvaro et al., 1992)

Studies in rats have demonstrated that the initial phase of the hypoxic response is mediated by the carotid body, is glutamate dependent and involves the nucleus tractus solitarius.(Ohtake et al., 1998) The response is increased following chronic intermittent hypoxia and is more marked in neonatal than adult rats.(Pawar et al., 2008)

The process underlying the hypoxic ventilatory decline is incompletely understood. The role of peripheral chemoreceptors in the decline phase is uncertain. Vizek et al. demonstrated in anaesthetised and awake cats, that carotid sinus nerve output increased in response to hypoxia and was sustained during the subsequent decline in ventilation. (Vizek et al., 1987) The authors concluded that the decline was, therefore, a central process. Further studies however in anaesthetised cats found that in some immature kittens the initial increase in carotid sinus nerve output in response to hypoxia was not sustained.(Carroll et al., 1993) While peripheral chemoreceptors may contribute the hypoxic decline is predominantly centrally mediated, as Dawes et al. demonstrated in lambs that section of the midbrain at the upper pons resulted in loss of the hypoxic roll-off.(Dawes et al., 1983) Furthermore in anaesthetised paralysed piglets with cut vagus nerves both an initial increase in respiratory activity as measured by electrical activity of a cut phrenic nerve was seen in response to hypoxia, and a later decline in respiratory activity, suggesting that the process is not dependent on intact peripheral chemoreceptors .(Lawson and Long, 1983)

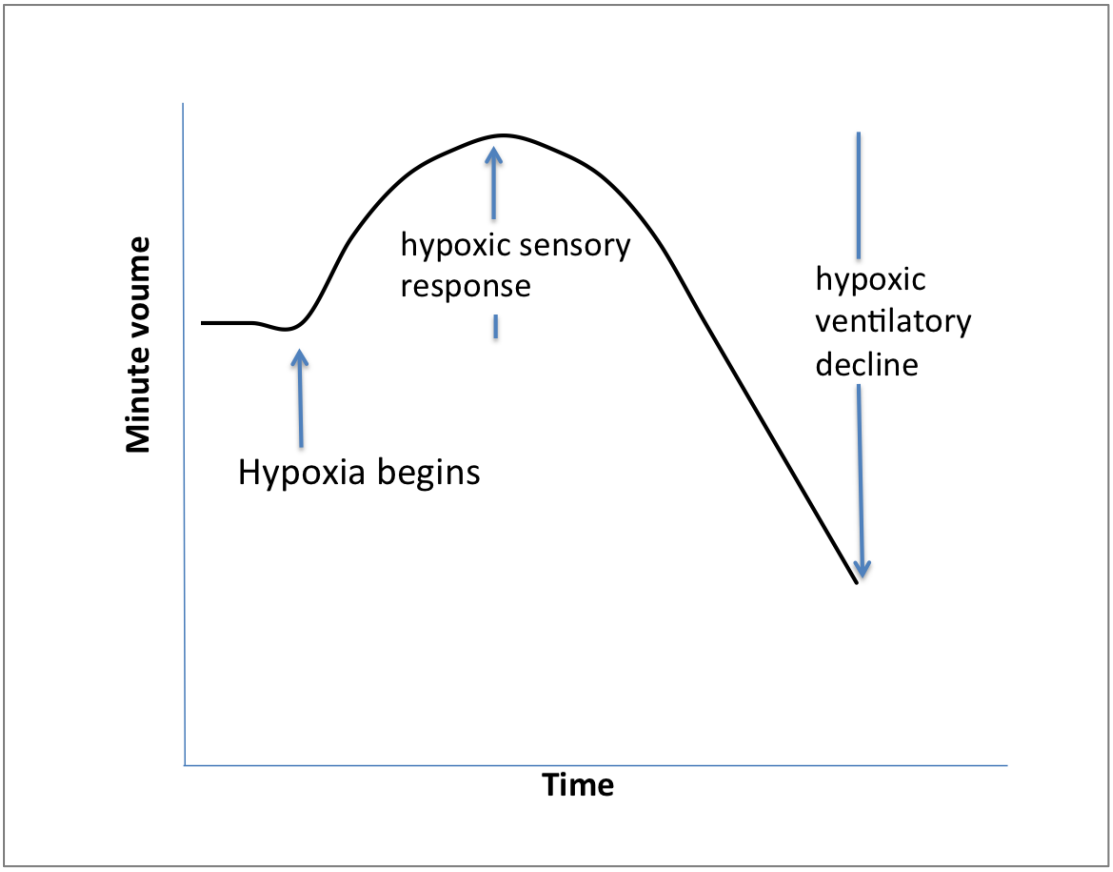


Figure 3: The biphasic response to hypoxia in newborn term infants: exposure to hypoxia results in an initial increase in minute ventilation (the hypoxic sensory response) followed by a late decline in minute ventilation (hypoxic ventilatory decline) (Cohen et al., 1997)

Numerous factors have been implicated in the hypoxic ventilatory decline including 5-HT,(Richter et al., 1999) adenosine,(Richter et al., 1999, Neylon and Marshall, 1991) nitric oxide,(Gozal et al., 1997) platelet derived growth factor(Simakajornboon and Kuptanon, 2005), opioid receptors (Kato et al., 2000) and γ-amino butyric acid (GABA).(Martin et al., 2004)

The roles of these factors in the hypoxic ventilatory decline are explored in detail by Simakajornboon and Kutpon in the table below.(Simakajornboon and Kuptanon, 2005) (Table 1)

Many of these pathways may be affected by in-utero exposure to smoking and substance misuse. Rats exposed to nicotine prenatally and in the neonatal period have reduced dopamine and increased tyrosine hydroxylase expression in the carotid bodies compared to non-exposed controls, suggesting perturbation of carotid body function.(Holgert et al., 1995, Gauda et al., 2001) Carbon monoxide may mediate an effect of maternal smoking on neonatal respiratory control. Carbon monoxide exposure inhibits carotid body firing in anaesthetised cats during exposure to hypoxia, (Lahiri et al., 1993) potentially by reducing hypoxic inhibition of potassium channels of chemoreceptors in the carotid body.(Riesco-Fagundo et al., 2001)
Opiates may have a direct effect on peripheral chemoreceptor function. Morphine inhibits spontaneous peripheral chemoreceptor discharge in cats.(McQueen and Ribeiro, 1980) Morphometric analysis of carotid bodies of adults who died of heroine overdose demonstrated histopathological changes compared to matched controls. However, these changes were comparable to those seen in chronic pulmonary disease. These changes may therefore be the result of recurrent hypoxia rather than the cause.(Porzionato et al., 2005)
Smoking and substance misuse may impair respiratory control either by a direct effect on the developing brainstem or by the induction of intermittent hypoxia by vasoactive components. (Kinney et al., 2009, Martin et al., 2004, Miller et al., 2000) This may be mediated by inducing intermittent hypoxia in the mother (Santiago et al., 1977) or potentially by disrupting placental blood flow inducing hypoxia in the fetus. (Woods et al., 1987)

Chronic hypoxia may alter the ventilatory response to hypoxia. The initial increase in ventilation in response to hypoxia is attenuated in rats exposed to sustained hypoxia in the

equivalent neonatal period,(Mayer et al., 2014) whereas exposure to sustained hypoxia at a later age results in an augmented response suggesting a vulnerable period of plasticity. The effect of sustained hypoxia and intermittent hypoxia on the hypoxic response may vary. Rats exposed to daily recurrent brief periods of hypoxia demonstrated an enhanced response to acute hypoxia, while exposure to a daily sustained period of hypoxia resulted in a diminished ventilatory response to hypoxia. (Peng and Prabhakar, 2004)

The hypoxic ventilatory decline is more marked in piglets that have been exposed to recurrent hypoxia and this is prevented by blocking GABA receptors with bicuculline, a specific GABA receptor blocker.(Martin et al., 2004) The expression of GABA receptors has been found to increase dramatically in rats at postnatal day 12 coinciding with an increased proportion of adult type receptors that may increase GABAergic transmission. This increased inhibition occurs concurrently with a precipitous drop in the excitatory neurochemicals glutamate and NMDA receptor subunits.(Wong-Riley et al., 2013) It has been shown that at this stage of development, postnatal day 12 in rats, the response to hypoxia is significantly impaired.(Liu et al., 2006)

The effect of chronic hypoxia on the ventilatory response to hypoxia may be a protective physiological response. The augmentation of the hypoxic response demonstrated in response to chronic intermittent hypoxia may demonstrate an adaptive response to an exogenous stressor. In contrast, the protective fetal response to hypoxia is primarily inhibitory, which is not protective post-natal. Enhancement of the hypoxic ventilatory decline by chronic hypoxia may therefore be considered a pathological response.

| **Neurotransmitters/modulators/effectors (location)** | **Role in respiratory control** | **Roles during development** |
|---|---|---|
| 1. GABA (caudal brainstem especially the nTS) | Hypoxic ventilatory depression. Inhibitory effect: tidal volume (GABA-α receptors) and frequency (GABA-β receptors) | Hypoxic ventilatory depression in developing animals |
| 2. Adenosine (caudal brainstem especially the rostral ventrolateral medulla) | Hypoxic ventilatory depression | Hypoxic ventilatory depression during early postnatal period and the effect decreases with maturation. Adenosine $A_{2A}$ receptors (hypoxic ventilatory depression); adenosine $_{A1}$ receptors (normoxia) |
| 3. Serotonin (5-HT) (caudal brainstem especially the hypoglossal nucleus and the rostral medulla) | Termination of early HVR through activation of 5-$HT_{1a}$ receptors. Excitatory (respiratory rhythm generator), inhibitory (hypoglossal nucleus) | Hypoxic ventilatory depression. 5-$HT_{1a}$ receptor density is high in the newborn period and decreases with maturation |
| 4. Opioids (caudal brainstem especially the nTS and the nucleus ambiguus) | Hypoxic ventilatory depression. Inhibitory effect: respiratory frequency (μ-opioid receptor), tidal volume (δ-opioid receptors) | Hypoxic ventilatory depression (μ-opioid receptors). μ-opioid and δ-opioid receptors increase with advancing age |
| 5. PDGF-β receptors (caudal brainstem especially the nTS) | Hypoxic ventilatory depression. Inhibitory effect through modulation of NMDA receptors | Important contributor of hypoxic ventilatory depression in young animals. Higher expression of PDGF-β receptors in the immature animals and expression decreases with increasing postnatal age. May provide protection against hypoxia-induced apoptosis |

Table 1: Neuromodulators implicated in hypoxic ventilatory decline (Simakajornboon and Kuptanon, 2005)

## 1.3 Risk factors for SIDS

SIDS remains the leading cause of death between the age of one month and one year in the developed world.(Blair et al., 2006) While SIDS is inherently a diagnosis of exclusion, the associated characteristics and risk factors for SIDS were sufficiently consistent for it to be recognised as a discrete condition. Numerous studies have been undertaken across the world with broad agreement of the recurring features and have led to a widely agreed definition as the sudden unexpected death of an infant (aged younger than 1 year), for which a post-mortem examination to an agreed protocol, and a review of the clinical history and circumstances of death failed to offer a sufficient explanation.(Blair et al., 2006) Several features consistently appeared among SIDS cases during the 1980s. Prone sleeping was the most prominent factor, alongside co-sleeping, prematurity, male sex, lower socio-economic group and maternal smoking. Furthermore a vulnerable time period became apparent. SIDS is rare in the first month after birth, with the incidence peaking at two to four months before becoming rare again beyond eight months of age. This combination of features led Wedgwood to suggest that SIDS would result from an overlapping of risk factors, (Wedgwood, 1972) this concept was later developed by Filliano & Kinney in the triple risk theory.(Filiano and Kinney, 1994)(Figure 4)

This theory suggested that there were inherent vulnerabilities within an infant, resulting from genetics, gender or antenatal and perinatal insults such as maternal smoking or prematurity, which at a critical time period made the infant vulnerable to an exogenous stressor.

### 1.3.1 Prone sleeping:

The predominance of prone sleeping as a factor for increased risk of SIDS led to intervention with the 'Back to sleep' (BTS) campaign that commenced in 1991. Prior to this intervention, the incidence of SIDS in England and Wales was around 2 in 1000 live births and 89% of infants who died of SIDS were found prone. Following the BTS campaign, the incidence had fallen to 0.26 in 1000 by 2003.(Blair et al., 2006) It has been suggested that prone sleeping may result in an increased risk of being smothered due to positional effect on the ability of the infant to clear the airway.(Tonkin, 1975)

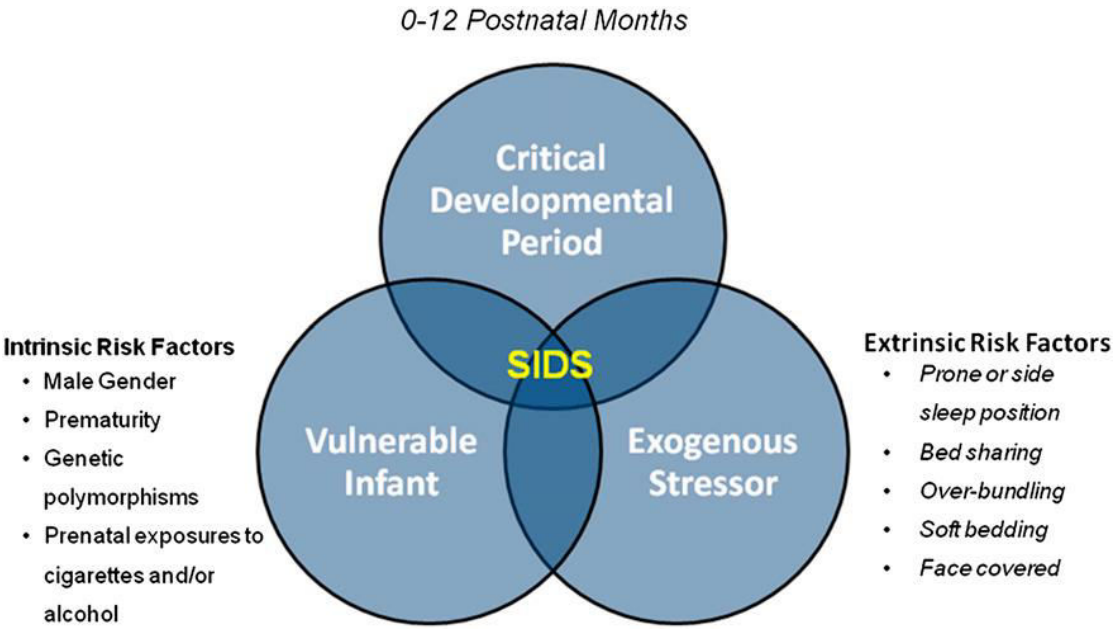


Figure 4: The triple risk theory for SIDS (Trachtenberg et al., 2012)

A counter argument is that the effect of prone sleeping in increasing vulnerability to SIDS is by a direct effect on sleep pattern and ventilatory responses.(Lewak, 2012).

The odds ratio for SIDS in preterm born infants have been reported as 2.57 for infants born between 24 and 28 weeks, 2.72 for those born between 29 and 32 weeks, and 1.85 for those from 33-37 weeks compared to term infants.(Malloy, 2013) The risks of prone sleeping are increased in prematurely born or 'small at birth' infants (born at <37 weeks gestation or birth weight <2500g), with an odds ratio of 140 for a small at birth infant sleeping prone compared to a supine sleeping infant who was not small at birth.

Franco et al. studied 25 healthy term infants at a median age of nine weeks when both sleeping prone and supine, exposing them to increasing auditory stimuli until arousal occurred. They found a significantly greater intensity of auditory stimuli was required to trigger arousal in the prone compared to the supine position.(Franco et al., 1998) Horne et al. evaluated arousal thresholds by applying air jets of varying intensity to the nares of sleeping healthy term infants. Arousal thresholds were significantly higher in the prone position when studied at two to three weeks and at two to three months of age. This difference had disappeared by five to six months of age.(Horne et al., 2001) Bhat et al., using polysomnography in the supine and prone position demonstrated the prone position was associated with greater sleep efficiency, with fewer arousals and more central apnoea.(Bhat et al., 2006)

Smith et al. studied the ventilatory responses to increasing $CO_2$ levels by assessing the pressures generated during an occlusion during the first 100 milliseconds ($P_{0.1}$), and the maximum pressure generated against an occlusion while crying (Pimax). They found the response was lower in the prone position.(Smith et al., 2010) Saiki et al. used increased dead space to evaluate the response to a primarily hypercarbic stress and found the time constant of the response was prolonged in the prone position suggesting a damped response to hypercarbia.(Saiki et al., 2010) Furthermore, the time constant increased with increasing post-menstrual age (PMA) towards the high risk period for SIDS but only in the prone position. Those studies support the hypothesis that prone sleeping is associated with a decreased respiratory drive, which may be a crucial component of the failure to autoresuscitate, resulting in SIDS.

The mechanism by which prone position may result in dampened ventilatory response remains unclear. Physiological studies in prematurely born infants have demonstrated improved oxygen saturation in the prone compared to the supine position at 36 weeks PCA.(Bhat et al., 2003, Baird et al., 1991)This was associated with no change in respiratory system resistance or compliance between positions, but significantly greater functional residual capacity (FRC) in the prone position.(Bhat et al., 2003) This benefit, however, was only statistically significant in oxygen dependent infants.(Kassim et al., 2007) To explore the effect of position on lung function at the high-risk age for SIDS, Saiki et al. measured oxygen saturation, FRC, compliance and resistance of the respiratory system in twenty infants born preterm both at neonatal unit discharge (median 36 weeks postmenstrual age) and 6 weeks later.(Saiki et al., 2009) They found significantly greater FRC in the prone position at both time points, suggesting that the increased risk of SIDS in the prone position does not reflect a mechanical disadvantage in this position. Pulmonary stretch receptors are some of the numerous afferents to the respiratory control centres and mediate the Hering-Breuer reflex. This vagally mediated reflex was described in 1868 by Hering and Breuer who demonstrated that lung inflation in anaesthetised cats resulted in termination of inspiration and prolonged expiration. In eighteen prematurely born infants (median gestational age 30 weeks) a stronger Hering-Breuer inflation reflex was demonstrated in the prone compared to supine position, which correlated strongly with the increased FRC in this position.(Landolfo et al., 2008)

**1.3.2 Maternal smoking:**

Prior to BTS, maternal smoking in pregnancy was recorded in 57% of SIDS cases, which increased to 86% of cases in England and Wales by 2003, making it the most important risk factor for SIDS.(Blair et al., 2006) Both smoking and substance-misuse are strongly associated with another risk-factor for SIDS, that is lower socio-economic group but it is thought that smoking and substance misuse in pregnancy may per se result in an intrinsic vulnerability in the infant.

It has been postulated that smoking during pregnancy may impair fetal neurodevelopment. This could be a direct result of hypoxia resulting from nicotine induced vasoconstriction,

(Suzuki et al., 1980) or as a result of abnormal distribution of neurochemicals. Autopsies of fetuses of smoking mothers aborted mid gestation showed increased nicotine binding sites in the tegmental nuclei, an area involved in cardiopulmonary integration, compared to controls. (Kinney et al., 1993)

Lewis et al. considered the arousal and ventilatory responses to hypercapnia and hypoxia in infants of smoking mothers, finding fewer awakenings in response to hypoxia in infants of smoking mothers. No difference in ventilatory response to either hypoxia or hypercapnia was demonstrated.(Lewis and Bosque, 1995)

Milner et al. compared the lung function of infants of mothers who smoked to controls. They found significant effects of antenatal exposure to smoking, which were specific to gender. Male infants had decreased static lung compliance when exposed to smoking, whereas female infants had impaired respiratory system resistance when exposed to smoking. Campbell et al. used a combined asphyxial challenge with increased $CO_2$ and decreased oxygen to measure ventilatory asphyxial sensitivity in infants of smoking mothers compared to controls. This was determined by comparing baseline minute ventilation with the last ten breaths of the challenge, which continued for up to five minutes.(Campbell et al., 2001) They found an increased ventilatory sensitivity to asphyxia in the smoking group. They concluded that a reduced ventilatory response in infants of smoking mothers was not the cause of an increased vulnerability to SIDS, however, the rate at which the infants responded to the asphyxia gas was not considered.(Milner et al., 1999) Horne et al. studied arousal thresholds using air jets and found an increased arousal threshold in infants of smoking mothers at two to three months of age, when the risk of SIDS is highest.(Horne et al., 2002) Bhat et al. used added dead space to determine the response to a predominantly hypercarbic challenge and found an increased time constant of the response in infants of smoking mothers, suggesting a damped response compared to controls.(Bhat et al., 2005)

**1.3.3 Maternal substance abuse:**

Substance misuse in pregnancy is common. An analysis of sequential positive urine pregnancy tests was performed for a range of substances, with 1 in 6 positive, although the

majority were for positive cannabis.(Sherwood et al., 1999) In a Spanish study hair analysis was performed on 209 pregnant women, which found evidence of substance misuse in 15.4% of mothers, with cocaine use most common (12.4%).(Lendoiro et al., 2013) Epidemiological studies have suggested a four to eight fold increased risk of SIDS in infants of substance abusing mothers (ISAMs) compared to controls,(Kandall et al., 1993, Ward et al., 1990) . A retrospective study looking at all SIDS cases in New York City over 10 years examined the effect of substance misuse on the rate of SIDS cases per 1000 live births. This study estimated the risk ratio of SIDS in infants of mothers who used methadone in pregnancy compared to non-exposed infants to be 6.95 (95% CI 4.94-6.8). (Table 2)

Klonoff-Cohen et al. highlighted an association between paternal cannabis use during pregnancy and subsequent SIDS. This was determined, however, by a telephone interview six months after the SIDS event that may reduce the validity of the results of the study (50). Determining how this effect occurs is confounded by factors associated with substance misuse such as low socioeconomic group, chaotic lifestyle and smoking. Furthermore the effect of different substances on the fetus is likely to vary as the pharmacological effects of the different drugs differ widely, and polypharmacy is common. In the study by Sherwood et al. 25% of positive urine drug screens from pregnant mothers were positive for more than one illicit substance.(Sherwood et al., 1999)

| | Births | SIDS deaths | Rate /1000 live births | Rate ratio (CI) |
|---|---|---|---|---|
| Total | 1,209,534 | 1760 | 1.45 | |
| No drug | 1,193,079 | 1664 | 1.39 | |
| Drugs | 16,455 | 96 | 5.83 | 4.19 (3.41-5.15) |
| Heroin | 1,805 | 12 | 6.65 | 4.78 (2.71-8.41) |
| Methadone | 3,416 | 33 | 9.66 | 6.95 (4.94-6.80) |
| Heroin, Methadone | 506 | 4 | 7.91 | 5.69 (2.14-15.12) |
| Cocaine | 8,868 | 41 | 4.62 | 3.32 (2.44-4.52 |
| Cocaine plus methadone or heroin or both | 1,860 | 6 | 3.23 | 2.32 (1.04-5.17) |

Table 2: SIDS deaths in New York City (January 1979 to December 1989) (Kandall et al., 1993)

**Critical period:**

49.1% of SIDS cases in England and Wales in 2012 occurred between the age of one and three months, with smaller proportions occurring in the first month and after 7 months (13%, 12% respectively).(2014) In a large study examining the age at death of prematurely born infants who succumbed to SIDS, Halloran et al. found that, with the exception of extremely prematurely born infants (<27 weeks gestation), prematurely born infants tended to die at an earlier post-conceptual age than term born infants.(Halloran and Alexander, 2006) The mean postnatal age at death for those infants was consistent across gestational age groups and was between 14 and 16 weeks. Those born at less than 27 weeks gestation died at a mean postnatal age of 20 weeks.(Halloran and Alexander, 2006) Saiki et al. noted that responses to tube breathing were increasingly damped towards the high risk period of 2-3 months.(Saiki et al., 2010) Fleming examined oscillations in ventilation following spontaneous sighs, and found in the newborn period that the responses were heavily damped and sluggish. Towards the high risk period the period of the oscillation shortened and the damping was reduced, resulting in a potentially unstable system, which subsequently stabilised into an appropriately damped adult type response by 6-8 months.(Fleming et al., 1984)

In a rat model, the expression of GABA receptors in the parafacial respiratory group and their transition to the rapidly responding adult type occurs at P12 which is roughly equivalent to the developmental stage of an infant at two to three months postnatal age.(Wong-Riley et al., 2013) At this time, there is also a significant down regulation of the serotonergic system(Liu and Wong-Riley, 2008), potentially moving the system towards a predominantly inhibitory state, reducing the ability of an infant to respond to an exogenous stressor.

### 1.3.4 Prematurity

The proportion of infants dying of SIDS born prematurely has increased in the United Kingdom following the BTS campaign, from 12% of cases between 1984-1988 to 34% from 1999-2003.(Blair et al., 2006) In the United States the odds ratio for SIDS in infants born at 24 to 28 weeks gestation compared to term born infants did not alter significantly between 1987 and 2005 (2.32 and 2.57 respectively).(Malloy, 2013) Halloran and Alexander retrospectively studied all SIDS deaths in the USA over a two-year period, and examined the effect of prematurity on timing of SIDS death.(Halloran and Alexander, 2006)

### 1.3.5 Gender

Male gender has been found to be a risk factor for SIDS, making up 56% of cases in England and Wales.(Blair et al., 2006) Despite this, no difference in ventilatory responses between male and female infants has been established. Animal studies, however, have shown differences in ventilatory control between genders. Studies of infant rats at the 'critical period' of P12-13 at which the response to hypoxia is markedly impaired showed significantly greater impairment in males than age matched females.(Holley et al., 2012) At this stage of development, male rats had siginificantly higher circulating levels of oestradiol than at other stages in development, and than matched female rats. It has been hypothesised that oestradiol may alter GABA receptor expression in respiratory neurons both altering ventilatory responses(Zuperku and McCrimmon, 2002) and increasing vulnerability to excititoxicity during hypoxia.(McCarthy, 2011)

Stress in the neonatal period has been shown to alter ventilatory responses in a sex specific manner in newborn rats. Following maternal separation the ventilatory response to hypoxia increased by 25% in male rats, but decreased by 30% in female rats, compared to controls that had not undergone maternal separation. (Genest et al., 2004) In contrast, the ventilatory response to hypercapnia was increased in female rats compared to controls, but reduced in male rats compared to controls.(Genest et al., 2007)

### 1.3.6 Infection

Infection has been implicated as a risk factor in SIDS and also as a factor in triggering apnoea in preterms. Olsson et al. hypothesised that infection would impair ventilatory responsiveness via a mechanism mediated by prostaglandin. To test this hypothesis they administered intraperitoneal IL-1β or Lipopolysaccharide (LPS), a strongly immunogenic molecule, to newborn rats. They found that both IL-1β and LPS resulted in a lower baseline respiratory rate, a poorer response to anoxia and greater mortality following anoxic challenge. These effects were negated by pre-treatment with the cyclo-oxygenase inhibitor Indomethacin, supporting their hypothesis that this process was prostaglandin mediated. In vitro studies, applied IL-1β to brainstem-spinal cord preparations showed no effect on respiration, whereas applying prostaglandin E2 inhibited respiratory activity. This supports the hypothesis that IL-1β

does not have a direct inhibitory effect on respiratory neurons, but is mediated by prostaglandins.(Olsson et al., 2003) Balan et al. went on to administer endotracheal LPS in rat pups and found that it resulted in increased expression of IL-1β in the medulla. This effect was reduced when the vagal nerves were transected. These pups had a reduced hypoxic ventilator response, which was not affected by carotid sinus nerve transection.(Balan et al.)Those study demonstrate that a systemic inflammatory response may result in a depressed respiratory response to hypoxia.

Epidemiological evidence to support a role for infection or inflammation as a risk factor for SIDS include (i) seasonal variation in the incidence of SIDS, (ii) viral infection occurring in the two week period prior to death has been reported in 44% of SIDS cases (Hoffman et al., 1988) and (iii) sleeping in a used mattress increased the risk of SIDS, particularly if the mattress came from another home,(Tappin et al., 2002) the latter suggesting that the mattress could provide a reservoir of infective agents.

There have been many studies seeking to establish whether particular pathogens result in SIDS or vulnerabilities in an infant that would cause susceptibility to common pathogens. Bettelheim et al. isolated a toxigenic strain of Escherichia coli (*E. Coli*) from the gastrointestinal tract of 21 of 46 SIDS victims, but not from the stool of any of 46 age matched living controls suggesting there may be a causal link.(Bettelheim et al., 1990) The same group went on to investigate blood and isolates from lung tissue finding a greater proportion of the lung isolates of SIDS deaths being positive for E Coli compared to deaths from other causes (28.8% vs 12.5%). In addition, blood cultures were positive for E. coli in 15.3% of SIDS cases but none of the controls.(Pearce et al., 1999)Further evidence to support either an infective or immune mediated process in SIDS was provided by studies by Sayers et al. who inoculated chick embryos with serum from infants who had died from SIDS, and controls. The serum was lethal from 10 of 11 SIDS infants but only 2 of 5 controls. (Sayers et al., 1999)

Nevertheless, the lack of any history, clinical picture or histopathological findings suggestive of overwhelming infection challenges the theory that SIDS can be attributed to a single infective cause. The results of several studies, however, have suggested that mild or even subclinical viral infection can potentiate bacterial toxicity, causing sub-lethal levels of toxin to become lethal.(Jakeman et al., 1991b, Jakeman et al., 1991a, Sarawar et al., 1994) Jakeman et al.

studied the effect of influenza infection on the $LD_{50}$ (the dose at which 50% of a population is killed by a toxin) of a series of bacterial toxins. Newborn rats were infected with influenza on day one after birth and concurrently treated with antibiotics to prevent bacterial superinfection. They were then inoculated with bacterial toxins on day five at varying doses. Influenza infection decreased the $LD_{50}$ 3-fold for staphylococcal γ toxin, 14-fold for staphylococcal α toxin, 84-fold for endotoxin and 219-fold for diphtheria toxin. They observed that when the pups died it was often in sleep without appearing clinically unwell. The authors therefore speculated that this process could contribute to the sudden death of an apparently healthy infant as occurs in SIDS. (Jakeman et al., 1991a)

It has been suggested that the increased risk associated with prone sleeping could increased the risk of infection, as bacterial nasal colonisation was found to be greater in the prone position.(Bell et al., 1996) The risk associated with maternal smoking has been attributed to nicotine exacerbating the lethality of bacterial toxins. Sayers et al. injected bacterial toxins from isolates from two SIDS victims into chick embryos, at various dilutions and in various combinations including with nicotine. They found that inoculation with nicotine increased the lethality of the bacterial toxin, causing non-lethal levels of toxin to become lethal.(Sayers et al., 1995)

Numerous factors increase the risk of SIDS, and many of these have been shown to have a direct effect on respiratory control. The risk of SIDS increases when these risk factors are present in combination. I hypothesise that SIDS results from a critical failure of respiratory control, and each risk factor may individually impact on respiratory control. This would account for the multifactorial aetiology demonstrated by epidemiological studies and developed into the triple-risk theory,(Trachtenberg et al., 2012) and the multiplicative increased risk of SIDS when those risk factors are present in combination.(Oyen et al., 1997) The most prevalent risk factor currently is maternal smoking in pregnancy and the risk of SIDS increases multiplicatively if this is combined with the infants sleeping prone. In this thesis I will examine the ventilatory responses to hypoxia and hypercarbia in infants exposed to maternal smoking and substance misuse in pregnancy, and explore how these responses are further affected by infant sleeping position.

## 1.4 Respiratory patterns in prematurity

### 1.4.1 Periodic breathing

Periodic breathing is an oscillatory respiratory pattern of series of short pauses. It is defined as at least three pauses of more than three seconds, interspersed by periods of breathing not longer than 20 seconds. While this is seen in healthy term infants, and adults under certain physiological conditions, it is more common in prematurely born infants. (Shannon et al., 1988) The incidence has been reported as approaching 100% in infants with a birth weight of less than 1kg,(Mathew, 2011) but is seen in 36% of infants of normal (>2.5kg) birth weight.(Fenner et al., 1973)

As periodic breathing is frequently seen in healthy term infants it has been suggested that periodic breathing should be considered a non-pathological phenomenon associated with prematurity. (Avery et al., 1963)

### 1.4.2 Apnoea

Apnoea is the cessation of respiratory airflow for an abnormal period of time. The definition has evolved over the last half century from a cessation lasting more than two minutes(Blystad, 1956) to a cessation of 20 seconds or more.(2003) By recognising apnoea as a cessation of respiratory airflow rather than a cessation of breathing it includes those obstructive apnoeas that occur with respiratory effort.

#### 1.4.2.1 Incidence of apnoea

Apnoea of prematurity is one of the most common reasons for the initiation of drug therapy in neonatal medicine.(Clark et al., 2006) The incidence of neonatal apnoea increases with decreasing gestational age, from 7% of those infants born at 34-35 weeks, to 54% in those born from 30-31 weeks post-conceptual age (PCA). (Henderson-Smart, 1981) The incidence is 90% in infants weighing less than 1000g.(Comer et al., 2001)

#### 1.4.2.2 Central Apnoea

Apnoea in the newborn period is strongly related to prematurity(Baird, 2004) and in the absence of other causative pathology is known as apnoea of prematurity (AOP). This condition usually resolves with increasing maturity and as such it has been suggested that it should be considered a developmental disorder reflecting physiological immaturity of respiratory control, rather than a disease state. (Abu-Shaweesh and Martin, 2008)

Central apnoeas are pauses in breathing not associated with any respiratory effort and therefore probably reflects insufficient drive from the brainstem respiratory centres. Any of the factors discussed earlier that reduce excitatory input to the respiratory centres, or increase inhibition, may therefore increase central apnoea.

Khan et.al. explored whether apnoea might result from oscillations of arterial $CO_2$ levels above and below an 'apnoeic threshold'. They demonstrated that end-tidal $CO_2$ levels dropped to a critical level prior to a pause and breathing resumed when end-tidal $CO_2$ returned to that level.(Khan et al., 2005) (Figure 4)

Describing this critical level as the 'apnoeic threshold' Khan went on to demonstrate that this threshold was closer to eupnoeic $CO_2$ levels in newborn infants than it is in adults. This would result in small fluctuations in $CO_2$ causing the level to drop below the threshold with resulting apnoea. This concept was also explored by Nakayama et al. who used pressure support ventilation in sleeping dogs to reduce the end-tidal $CO_2$ to the point when apnoea, occurred. This was assessed during metabolic acidosis, alkalosis, hypoxia and administration of almitrine – a non-hypoxic peripheral chemoreceptor stimulant.

The authors concluded that, with the exception of hypoxia, all factors which increased respiratory drive also widened the gap between eupnoeic $CO_2$ levels and the apnoeic threshold, thus protecting against periodic breathing and apnoea. Conversely metabolic alkalosis, which blunts respiratory drive, narrowed that gap. (Nakayama et al., 2002)

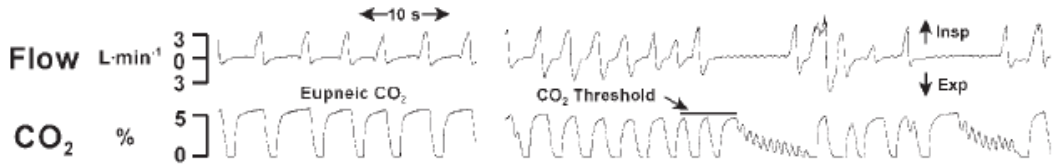


Figure 5: The apnoeic threshold.respiratory traces showing airflow and $co_2$ levels prior to and following apnoea. apnoea ensues when the end-tidal $co_2$ level drops below the 'apnoeic threshold'. (Khan et al., 2005)

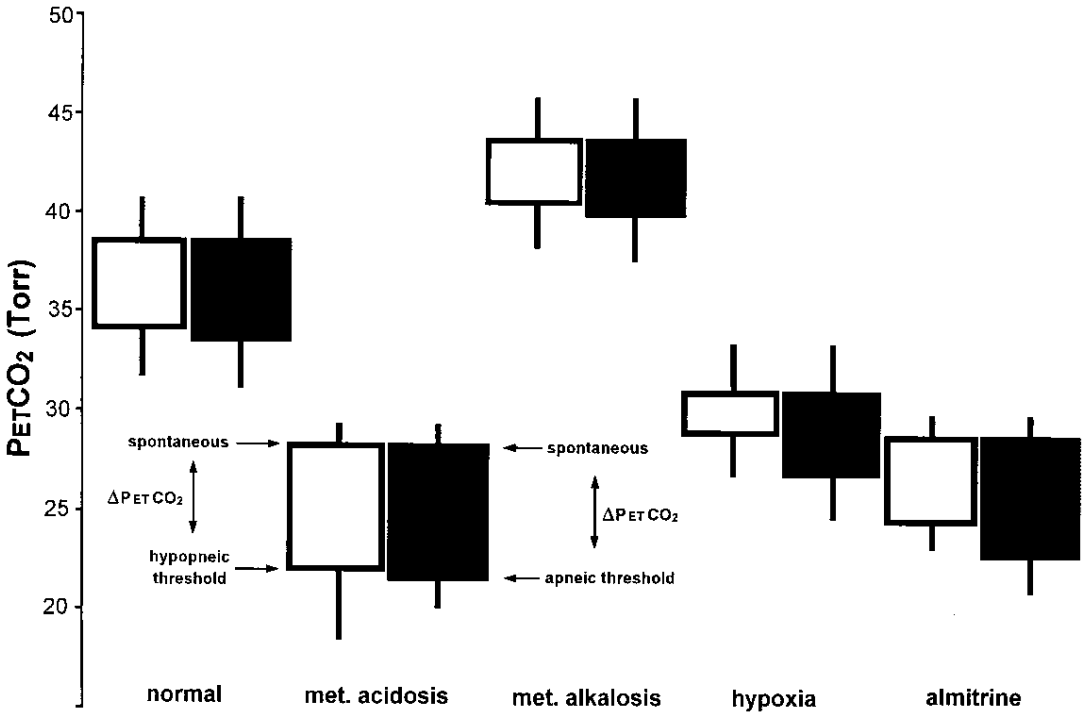


Figure 6: Mean end-tidal $CO_2$ of dogs during NREM sleep while breathing spontaneously (upper border of white and black boxes, whisker showing ± SD), and level at which hypopnea (lower border of white box) or apnoea (lower border of black box) occurs, under differing conditions. The gap between spontaneous breathing end-tidal $CO_2$ and apnoeic threshold $CO_2$ is widened by all respiratory stimulants, reducing the risk of apnoea, except hypoxia which narrows the gap thus increasing the susceptibility to periodic breathing and apnoea. (Nakayama et al., 2002)

Rigatto et al. examined the effect of prematurity on the ventilatory response to inhaled $CO_2$ in nine preterm infants. Using a steady state technique, where the infant is exposed to a controlled level of $CO_2$ for a prolonged period, they demonstrated an increasing sensitivity to $CO_2$ with increasing postnatal age. Furthermore they compared the sensitivity on day five after birth in a group of term infants and preterm infants (median 32 weeks of gestational age) and found that those born at an earlier gestation had a reduced sensitivity at the same postnatal age.(Rigatto et al., 1975) They hypothesised that an impaired sensitivity to $CO_2$ could account, at least in part, for periodic breathing and apnoea. In keeping with this theory it has been observed that periodic breathing stabilises and apnoea reduces with increased inspired $CO_2$.(Al-Saif et al., 2008)

Another component to this hypothesis is the role of damping within the respiratory control system. Fleming et.al explored the oscillations in respiratory pattern following spontaneous sighs.(Fleming et al., 1984) They found that following a sigh the response in the first four days of life was sluggish and over-damped with a slow return to normal breathing. This changed to a shorter period, poorly damped oscillation from the first week after birth until two to three months of age, when the system stabilised into the appropriately damped adult type response. An unstable oscillating system would be more likely to drive $CO_2$ levels below the apnoeic threshold resulting in periodic breathing and potentially apnoea. This is in keeping with the observation that periodic breathing rarely occurs in the first few days of life in the preterm infant.(Fenner et al., 1973)

#### 1.4.2.3 Obstructive & mixed apnoea

Obstructive apnoea is defined as a cessation of respiratory airflow with continued respiratory movements. The proportion of apnoeas that are purely obstructive has been reported at between 6.5 and 12%, but a far greater proportion of apnoeas have been shown to have at least one respiratory effort without successful generation of airflow, and therefore to have an obstructive element. These are described as mixed apnoeas.(Butcher-Puech et al., 1985). By monitoring the presence of the cardiac artefact, a fine oscillatory airflow generated in the airway by cardiac contraction, attenuated by increased airway resistance and absent in upper airway obstruction, Milner et al, however, demonstrated airway occlusion in up to 40% of

central apnoeas which increased with apnoea duration, reaching 100% in apnoeas of more than 20 seconds duration.(Upton et al., 1992b)

Evidence for the level of obstruction has been gathered using manometric studies to measure airway pressure at different anatomical levels. These studies have supported the hypothesis that the obstruction is in the upper pharynx, and potentially a result of reduced pharyngeal muscle tone in an already compliant structure.(Mathew et al., 1982) However, when fibreoptic microlaryngoscopy has been used to observe the laryngeal structures during apnoea, it was noted that during obstructive and mixed apnoeas there was laryngeal closure and subsequent movement of the laryngeal structures as a result of respiratory effort against the obstruction.(Ruggins and Milner, 1991) This would suggest that obstruction could be occurring at different levels, with laryngeal movement sufficient to cause the pharyngeal airway pressure changes detected in manometric studies. Prior to glottic closure pooling of secretions in the piriform fossa was observed, which may be a causative factor in laryngeal closure, and the induction of apnoea via the laryngeal chemoreflex.(Davies et al., 1988, Thach, 2010)
It has been shown that airway occlusion in itself can induce central apnoea,(Milner et al., 1977, Cohen and Henderson-Smart, 1986) and it may be that this sequence of events is responsible for a proportion of mixed apnoeas.

### 1.4.3 Resolution of apnoea of prematurity

Apnoea of prematurity invariably resolves with maturation and has usually resolved by 36 weeks postmenstrual age. In the extremely preterm babies, however, it may persist beyond term.(Eichenwald et al., 1997) Henderson-Smart et al. noted that the conduction of brainstem auditory evoked potentials (BAEP) were slower in preterm babies with apnoea and improved as the apnoea improved. They suggested that this was a result of slow brainstem conduction which resolved with myelination, and hypothesised that apnoea of prematurity may resolve with brainstem myelination.(Henderson-Smart et al., 1983) It has been suggested that the delay of resolution of AOP in extremely preterm babies reflects delayed myelination.

### 1.4.4 Long term effects of neonatal apnoea

There are limited data to determine the impact of apnoea of prematurity on long-term outcome, as it is particularly difficult to dissociate the effects of other insults in the neonatal

period. Pillekamp et al. scored the severity of apnoea and bradycardia, and the age at which it was occurring, and correlated this with neurodevelopmental outcomes at 13 months. They found there was a poorer neurodevelopmental outcome when there was more severe apnoea and bradycardia during a key developmental period of 31-37 weeks PMA, and when there was persistence of apnoea and bradycardia beyond term.(Pillekamp et al., 2007) Cheung et al. performed pneumograms on 164 very low birth weight infants prior to discharge, at a post conceptual age of over 35 weeks, and found an association between pre-discharge apnoea and poorer motor and mental neurodevelopmental scores at follow-up at two years of age.(Cheung et al., 1999) Koons et al. compared the outcome of sixty preterm infants with apnoea at neonatal unit discharge with the outcome of forty seven gestational age, weight and severity of illness matched infants free of apnoea at time of discharge, and found no difference in neurodevelopmental outcome at follow-up at one to two years age between the two groups.(Koons et al., 1993) While the authors concluded that there was no association between persistent apnoea and poorer neurodevelopmental outcomes compared to those in whom neonatal apnoea had resolved prior to discharge, the study was not designed to assess the effect of neonatal apnoea per se.

Although the effect of apnoea of prematurity on long term outcomes is uncertain and difficult to assess, it is physiologically plausible that recurrent hypoxic episodes resulting from apnoea may be detrimental to the infant, and efforts have been made to limit neonatal apnoea.

### 1.4.5 Methylxanthines

The mainstay of treatment for apnoea of prematurity has been methylxanthines: initially theophylline and aminophylline and subsequently caffeine, which have been shown in a meta-analysis to reduce apnoea frequency and need for positive pressure ventilation two to seven days after commencing therapy.(Henderson-Smart and Steer, 2001) Methylxanthines are phosphodiesterase inhibitors and at therapeutic concentrations non-specific adenosine receptor antagonists(Fredholm et al., 1999). Methylxanthines may affect respiratory control via adenosine receptors, either directly or by increased expression of adenosine receptors in the ventrolateral medulla and the rostral dorsolateral pons, as has been shown in the rat.(Gaytan et al., 2006)

Adenosine triphosphate (ATP) is cleaved to produce adenosine di-phosphate (ADP) and adenosine monophosphate (AMP); this in turn is converted to adenosine. In periods of hypoxia when reuptake of ADP and production of ATP is limited, adenosine will accumulate and may be critically placed to mediate hypoxic responses. While adenosine may play a role in the respiratory response to hypoxia, it has also been implicated in a direct neuroprotective response to hypoxia. It is thought that it may stabilise neurons, reducing neuronal firing and excitotoxicity.(Hagberg et al., 1987)In adult brain activation of $A_1$ receptors has been shown to be neuroprotective in ischaemia, that effect is blocked by caffeine.(Fredholm, 1995) However chronic caffeine consumption in adults has been suggested to be protective against neurodegenerative disease.(Fredholm, 1995) Furthermore, $A_1$ receptor agonists induced periventricular white matter changes in neonatal rats which were blocked by adenosine receptor antagonists.(Turner et al., 2002) Those studies all raised uncertainty over possible risks or benefits of the use of methylxanthines in neonatal practice. Further studies demonstrating that caffeine decreases cerebral perfusion, while increasing brain oxygen consumption,(Chen and Parrish, 2009) contributed to concerns that caffeine may contribute to poorer neurodevelopmental outcomes. In the fetus the protective response to hypoxia is to minimise metabolic demands, and this may be mediated by activation of adenosine receptors. Developmental changes in the expression of adenosine receptors may contribute to the evolution of ventilatory responses from fetal to neonatal life, and the persistence of fetal type receptors in the preterm infant may contribute to the immature respiratory pattern. However, adenosine may also mediate the excitatory protective mechanism in the term infant. Use of adenosine receptor blocking methylxanthines such as caffeine to stabilise respiratory pattern in prematurely born babies may therefore also block important protective mechanism for life ex-utero.

The various and apparently conflicting results from animal studies examining the effect of adenosine and adenosine receptor antagonists on the developing brain drove the need for a randomised controlled trial to evaluate the long term effect of caffeine in the treatment of neonatal apnoea.

The caffeine for apnoea of prematurity (CAP) trial was undertaken to determine the short and long term efficacy and safety of caffeine treatment for apnoea of prematurity. The eligibility

criteria were infants with birth weight of between 500g and 1250g, whom the clinician deemed a candidate for methylxanthine therapy in the first ten days of life. Two thousand and six preterm infants were randomised to receive either caffeine or placebo. Caffeine therapy was associated with a significant reduction in bronchopulmonary dysplasia (BPD), shorter duration of respiratory support and fewer infants requiring surgical closure of a patent ductus arteriosus (PDA). There was no difference in mortality.(Schmidt et al., 2006) Follow-up at 18-21 months of age showed a significant reduction of neurodevelopmental disability in the group that received caffeine,(Schmidt et al., 2007) although this difference was no longer significant when the children were followed up at five years of age.(Schmidt et al., 2012) This study provided reassurance that the potentially detrimental effects of adenosine receptor blockade were not clinically significant.

#### 1.4.5.1 Mechanism of action of methylxanthines

Several studies have explored the effect of methylxanthines on the ventilatory response to $CO_2$. Various techniques have been employed including rebreathing, where expired $CO_2$ gradually increases the inspired $CO_2$ concentration and is plotted against the coinciding increase in minute volume. This is a relatively simple and quick technique, but assumes that the maximal ventilatory response is achieved immediately at each point.(D'Urzo et al., 1990) The steady state technique maintains a constant inspired $CO_2$ level for a period sufficient to achieve a maximal response (usually five minutes).(Rigatto et al., 1975, Davi et al., 1978) This allows both the dynamics and the magnitude of the response to be assessed. Mazzarelli et al. found an increased sensitivity to inspired $CO_2$ in anaesthetized cats after administration of caffeine using a steady state technique.(Mazzarelli et al., 1986) Similar findings were reported in a small study using rebreathing in adult humans following oral caffeine.(D'Urzo et al., 1990) Davi et al. studied the effect of intravenous theophylline on preterm infants being treated for apnoea of prematurity, and again found an increased ventilatory response to inspired $CO_2$ using the steady state. (Davi et al., 1978) Pianosi employed both techniques while studying the effect of caffeine on sensitivity to $CO_2$ in a group of young adults to determine if the different techniques gave differing results. While both techniques found an increased sensitivity to $CO_2$ following administration of caffeine, Pianosi found that using a rebreathing technique highlighted that caffeine had an additive effect on ventilation at a given $CO_2$ level, while steady state measurement demonstrated a multiplicative effect on minute volume.

Caffeine therapy has also been shown to have an effect on respiratory muscle function. In a study of preterm infants being weaned from mechanical ventilation caffeine therapy was associated with an increased Pimax and Pemax (maximal inspiratory and expiratory pressures generated), and improved functional residual capacity and respiratory system compliance and resistance.(Kassim et al., 2009)

**1.4.6 Summary:**

Apnoea is a common problem on the neonatal intensive care unit, and caffeine a widely used therapy. However, the effect of prematurity on the mechanisms of respiratory control, and how this is modified by caffeine therapy, remains incompletely understood. The response to carbon dioxide may be a critical factor in developing stable respiratory control. In this thesis I will use the steady state technique, in which the infant is exposed to a controlled steady level of inspired carbon dioxide, to measure the ventilatory response to hypercarbia in preterm infants, test the hypotheses that those infants that go on to develop apnoea will have a lower ventilatory response to hypercarbia in the newborn period, the response to hypercarbia will increase with maturity, and caffeine therapy.

## 1.5 Gastro-oesphageal reflux and apnoea

### 1.5.1 Incidence

Gastro-oesophageal reflux (GOR) is a frequent phenomenon in infants and when associated with morbidity is called gastro-oesophageal reflux disease (GORD). GORD appears to be common. 20% of infants in Belgium received a prescription for either a proton pump inhibitor or histamine receptor blocker (Vandenplas, 2013). In the preterm population GORD may be a greater problem. Of 1598 extremely low birth weight (<1000g) infants admitted to NICU in the USA 24.8% were discharged on anti-reflux medication. Of those discharged post term (>42 weeks post menstrual age) this rose to 47.6%.(Malcolm et al., 2008)

### 1.5.2 Reflux and apnoea

Both gastro-oesophageal reflux and apnoea are common in preterm infants. Menon et al. studied nine preterm and one term infant with vomiting and apnoea to determine if the two were associated. They found an increased frequency of apnoeas immediately following regurgitation compared to a regurgitation free control period. The authors suggested a potential mechanism for this association between regurgitation and apnoea in the laryngeal chemoreflex.(Menon et al., 1985) The laryngeal chemoreflex (LCR) was first described in 1975 with a study in which water was introduced into the larynx of newborn lambs inducing prolonged apnoea and bradycardia; saline had no effect.(Harding et al., 1976) It was determined that chemosensitive receptors particularly concentrated in the mucosal epithelium of the aryepiglottic folds, interarytenoid space and epiglottis mediated the reflex.(Boggs and Bartlett, 1982) The afferents that mediate the LCR are unencapsulated, unmyelinated nerves that form the superior laryngeal nerve, which respond to the ionic content of fluid bathing the overlying mucosa. Some laryngeal afferents in the superior laryngeal nerve synapse directly with cardiac vagal neurons in the nucleus ambiguous, while others terminate in the solitary tract nucleus. Rapid inhibition of the phrenic and vagal motor neurons may account for the immediate onset of apnoea and bradycardia in the LCR.(Mendelowitz, 2000) The strength of the response to introduction of liquid was related to the concentration of chloride, but also triggered by strong acid or alkali irrespective of chloride concentration.(Boggs and Bartlett, 1982)The response to stimulation was rapid, and involved swallowing, apnoea, bradycardia,

hypertension and often stridor, along with arousal and occasional coughing. The response was dependent on maturity; prematurely born babies predominantly displaying prolonged apnoea, bradycardia and stridor with few coughs, whereas term babies had fewer swallows, shorter apnoea and increased cough and arousal.

Factors that exacerbate the reflex include anaemia, hypoxia and upper airway infection. Thach suggested that enhancement of the LCR by hypoxia may be beneficial in preventing aspiration of meconium or amniotic fluid during hypoxia at birth, and enhancement by viral infection may help prevent aspiration of upper airway pathogens causing bacterial pneumonia.(Thach, 2010) The LCR is a plausible mechanism by which reflux could precipitate apnoea in the preterm infant. This is supported by the observation of Ruggins et al. who performed microlaryngobronchoscopy on infants with apnoea, and noted that pooling of secretions in the piriform fossa often preceded apnoea.(Ruggins and Milner, 1991)

### 1.5.3 Treatment of reflux

Non-pharmacological management of reflux is largely focused on positioning of the infants after feeds. Vandenplas found significantly less acid reflux in the prone position compared to the supine in asymptomatic infants on the neonatal unit.(Vandenplas and Sacre-Smits, 1985) Bhat et al. found a similar increase in reflux index in the supine compared to the prone position in asymptomatic prematurely born infants,(Bhat et al., 2007)A randomised control trial of elevation of the head by $30^{o}$ found no improvement of reflux in symptomatic infants,(Orenstein, 1990) and placing in an infant seat at an angle of $60^{o}$ worsened GOR.(Orenstein et al., 1983)

Feed thickeners such as rice cereal or carob have been used in the treatment of reflux for several decades. Studies have shown that they may reduce the incidence of vomiting without altering the reflux index.(Vandenplas and Sacre, 1987, Bailey et al., 1987, Wenzl et al., 2003) Furthermore thickeners have been shown to increase coughing compared to unthickened feeds.(Orenstein et al., 1992) Alginates work by forming a floating foam over the gastric contents, and have been shown to reduce the oesophageal height of reflux episodes, and reduce acid reflux, while having no effect on the number of non-acid reflux events.(Corvaglia et al., 2011a)

Prokinetics have been used extensively in the management of infant GORD; nearly one in five infants admitted to NICU in the USA were commenced on Cisapride during admission in the 1990s.(Ward et al., 1999) This prokinetic works, as a serotonergic receptor agonist, stimulates the release of acetylcholine at the myenteric plexus.(Hegar et al., 2009) It has been shown to reduce the reflux index, (Augood et al., 2003)but not to improve symptoms of GOR.(Maclennan et al., 2010) Cisapride was withdrawn from use, apart from under the supervision of a paediatric gastroenterologist, in the US and Europe in 2000 due to reports of fatal cardiac arrhythmias.(Maclennan et al., 2010) Other prokinetics with limited evidence of efficacy have been used such as metoclopramide (a dopamine receptor blocker), erythromycin or domperidone. Metoclopromide is rarely used in infants largely due to a side effect profile that includes methaemaglobinaemia, arrhythmias, and neuroendocrine effects such as galactorrhoea.(Vandenplas et al., 2005) Erythromycin, a macrolide antibiotic, is thought to work as a prokinetic by increasing migrating motor complex activity resulting in gastrointestinal contractions.(Curry et al., 2001)Potential side effects include arrhythmia and pyloric stenosis.
Domperidone is a peripheral dopamine $D_2$-receptor antagonist thought to increase oesophageal motility and gastric emptying.(Reyntjens et al., 1978) The efficacy of domperidone remains uncertain, with conflicting results from several placebo controlled trials.(Pritchard et al., 2005) Its efficacy was compared to Cisapride in a randomised trial of 20 symptomatic infants and found equivalent to Cisapride in reducing regurgitation, although the authors noted that the improvement in both groups might reflect the natural evolution of GORD over time.(Hegar et al., 2009) A recent systematic review (Pritchard et al., 2005) to determine the efficacy of domperidone in the treatment of GOR concluded that there was insufficient evidence to support its use in uncomplicated GOR.

Antacids and acid suppressants have been used to increase the pH of reflux to reduce symptoms and acid related pathology. Antacids include a range of alkali formulations that neutralise gastric pH rapidly and commonly include aluminium and magnesium containing salts, which may induce diarrhoea, osteopenia and neurotoxicity, and as such their use in infants is limited. Some alginates have a significant level of alkali in their preparation. Alginate(s) were shown to reduce the indices of acid reflux, but not non-acid reflux, in a

placebo controlled crossover trial in infants with apnoea of prematurity.(Corvaglia et al., 2011a)

$H_2$ receptor antagonists and proton pump inhibitors are used to increase gastric pH by inhibiting the secretion of gastric acid. Ranitidine is the $H_2$ receptor antagonist commonly used in the treatment of GORD in neonatal units. While it has been shown to be effective in the treatment of erosive oesophagitis in children,(Cucchiara et al., 1993) there is an absence of efficacy data for the use of ranitidine in the treatment of GORD in infants.(Vandenplas et al., 2005) In a prospective multicentre observational study including 274 infants of 24 to 32 weeks gestation or birth weight between 401 and 1500g Terrin et al. demonstrated a significantly increased risk of bacterial infection, necrotising enterocolitis and death in infants that received Ranitidine compared to those that did not receive Ranitidine.(Cothran et al., 1997)There is evidence that tolerance to Ranitidine can occur, with reports of gastric pH returning to baseline levels within as little as three days in adults.(Lachman and Howden, 2000)pH and endoscopy investigation of 103 infants with persisting reflux symptoms despite treatment with ranitidine revealed oesophagitis and high reflux indices suggesting that such tolerance may also occur in infants.(Salvatore et al., 2006) Proton pump inhibitors such as omeprazole have been shown to be effective in suppressing gastric acid production (Faure et al., 2001)and an effective treatment for reflux oesophagitis.(Cucchiara et al., 1993) Working by irreversibly blocking the parietal cell $H^+/K^+$ ATPase they have a longer half-life than $H_2$ receptor antagonists allowing once daily dosing.(Vandenplas et al., 2005) While the efficacy of omeprazole in reducing oesophageal acid exposure in infants has been demonstrated,(Omari et al., 2007) symptomatic improvement has not been shown.(Moore et al., 2003, Orenstein et al., 2009)In a double-blinded randomised placebo controlled trial lansoprazole was no more effective than placebo in treating symptoms attributed to gastro-oesophageal reflux, but was associated with significantly more serious adverse events than placebo of which the most frequent was lower respiratory tract infection. Similarly to $H_2$ receptor antagonists, there are also concerns about side effects relating to overgrowth of gastrointestinal flora. Cothran et al. studied gastric pH and gastric colonisation of eighty-six preterm infants, twenty-three of whom received ranitidine therapy. Ranitidine therapy was associated with a higher gastric pH and increased gastric colonisation with pathogenic bacteria.(Cothran et al., 1997)

Despite concerns regarding efficacy of anti-reflux medication, and potential effects of altering gut flora in the infant, anti-reflux medication is widely used, often with the intention of reducing apnoea. Effective identification of those infants with reflux related apnoea would avoid unnecessary and ineffective treatment and potential gastro-intestinal complications.

### 1.5.4 The effect of reflux treatment on apnoea

Several studies have evaluated the effect of reflux treatment on cardio-respiratory events such as apnoea or bradycardia in the neonatal period. Corvaglia et al. evaluated the effect of sodium alginate (Gaviscon) on the frequency of apnoea in 28 preterm infants, and found no significant difference between those receiving placebo or Gaviscon.(Corvaglia et al., 2011a) Wheatley et al. examined the effect of metoclopramide and ranitidine compared to placebo on the frequency of bradycardia episodes in a masked cross-over trial and found no significant differences.(Wheatley and Kennedy, 2009) They did, however, note that the frequency of apnoea reduced significantly in both groups with time and emphasised the importance of accounting for this in future study design. In a retrospective study Misra et al. examined the frequency of apnoeas in infants with apnoea and evidence of reflux before and after commencing transpyloric feeding. They found an improvement in 12 of 15 infants after forty-eight hours.(Misra et al., 2007) There was, however, no means to account for the improvement with time noted by Wheatley. Newell et al. evaluated GOR using pH studies in very low birth weight infants and in a subgroup of infants with xanthine resistant apnoea found treatment of reflux was associated with resolution of apnoea.(Newell et al., 1989) Again the study did not control for the natural resolution of apnoea with time.

Whether treatment with gastro-oesophageal reflux improves apnoea remains uncertain. Failure of GORD treatment to improve apnoea may reflect inefficacy of medication, or lack of causation between reflux and apnoea.

### 1.5.5 The association between reflux and apnoea

Early techniques using pH probes failed to show a temporal association between reflux episodes and apnoea,(Menon et al., 1985, Arad-Cohen et al., 2000) but as they would only detect acid reflux, this would not detect non-acid reflux which could still be a potent trigger of the LCR. Peter et al. used multiple intraluminal impedance (MII) techniques to assess reflux

irrespective of pH, but found that there was no evidence that reflux episodes preceded apnoea in nineteen infants with a diagnosis of apnoea of prematurity. They noted that there was an increase in reflux episodes after apnoea, and postulated that this may be as a result of apnoea induced loss of tone, including that to the lower oesophageal sphincter, predisposing to reflux episodes. (Peter et al., 2002) In contrast, Corvaglia et al. used combined MII and pH probes to assess the relationship between reflux episodes and apnoea in 58 preterm infants with recurrent apnoeas. They found a statistically significant association between reflux episodes and apnoea, but that was because there was a strong temporal relationship in a subset of the population, whereas the remainder of the population showed no association. The subgroup was indistinguishable from the remainder of the population.(Corvaglia et al., 2011b) Nunez et al. studied the temporal relation between reflux and apnoea in a small group of ex-preterm infants who continued to have apnoea at or after term, and again found a statistically significant association between reflux and apnoea in a small subset of the study population. (Nunez et al., 2011) These studies suggest that there is a small proportion of preterm infants for whom GOR may be a cause of apnoea. Apnoea is a common problem on the neonatal unit, and successful identification of those infants in whom reflux may contribute to apnoea, and therefore may benefit from treatment of reflux is essential. In this thesis I will seek to determine if there is an association between reflux episodes and apnoea, and characterise those apnoea events that are likely to be associated with reflux. This may identify those infants for whom reflux treatment will be beneficial.

### 1.5.6 Investigation of reflux

Several techniques have been used to assess reflux. Upper gastrointestinal barium contrast radiography has been shown to be neither sensitive nor specific in detecting reflux, but useful in determining anatomical abnormalities.(Rudolph et al., 2001) Endoscopy and biopsy allows for evidence of chronic reflux such as oesophagitis, but cannot be used to diagnose reflux directly. Nuclear scintigraphy has been used and while specificity has been reported from 83-100%, sensitivity in detecting reflux is only between 15 and 59%.(Seibert et al., 1983)

#### 1.5.6.1 pH Studies

For many years the gold standard for detection of GOR has been 24 hour pH monitoring.(Vandenplas et al., 2005) Using a pH sensitive probe in the lower oesophagus, the

time that oesophageal pH drops below a threshold of pH 4 can be quantified. While this “reflux index”, the percentage of the study time with pH <4 is used to diagnose abnormal, the cut-off used has varied from 5%(Aksglaede et al., 2003) to 8%.(Vandenplas et al., 1991) Other parameters used in the evaluation of pH studies include the total number of episodes where pH <4 in 24 hours, the number of which last >5 minutes and the duration of the longest episode in minutes. Attempts have been made to improve the sensitivity of pH monitoring by developing composite scores, such as the Boix-Ochoa composite score which incorporates the parameters above weighted according to body position (i.e upright or recumbent), to determine those infants requiring treatment.(Boix-Ochoa et al., 1980) pH studies are limited as they can only detect acid reflux. In infants on frequent feeds the gastric pH may drop below 4 for as little as 10% of the time, therefore 90% of reflux episodes in these infants would be missed by pH monitoring.(Grant and Cochran, 2001) As non-acid reflux may cause symptoms the inability of pH study to detect these events may limit its use particularly in determining symptom association.

#### 1.5.6.2 Multichannel intraluminal impedance and pH

During multichannel intraluminal impedance (MII) monitoring the resistance to an alternating current is detected between a series of channels along the length of the probe. When liquid bridges the gap between channels the impedance drops and hence the technique can detect reflux events irrespective of pH. Incorporation of a pH probe with MII assessment allows detection of all reflux episodes and classification of these episodes by pH. This technique is now considered the gold standard for detection of reflux,(Vandenplas et al., 1991) but is limited to some extent by the challenges in recognising and scoring reflux events. Di Fiore et al. found that 59% of acid-based reflux events detected by pH probe were not detected by impedance monitoring as a result of not meeting scoring criteria, technical artefact, positive impedance deflections or no change in impedance.(Di Fiore et al., 2009) Furthermore there are few normal data in infants.(Wenzl, 2003, Lopez-Alonso et al., 2006) In this thesis I will use pH/MII to assess reflux events capturing both acid and non-acid reflux capable of inducing apnoea.

Several studies have evaluated the association between reflux events and apnoea with inconsistent results. Many of these have been limited by the use of pH in isolation, missing the possible effect of non-acid reflux. While there are plausible mechanisms by which reflux may induce apnoea the association has not been clearly demonstrated. Furthermore while reflux may induce apnoea, the converse is also true: the frequency of reflux is higher after prolonged apnoea possibly resulting from loss of oesophageal tone during hypoxia. This may mask any association between apnoea and reflux if one compares the frequency of apnoea before and after reflux events.

It remains debatable whether gastro-oesophageal reflux may cause apnoea, or if medical management results in any improvement. In this thesis I aim to evaluate variation in practice in the investigation and management of GORD on neonatal units in the United Kingdom, evaluate the use of multichannel intraluminal impedance in conjunction with pH study to detect GORD and compare this technique to the upper GI contrast study in detecting GORD, and determine if a proportion of apnoeas result from reflux and in which infants this association may be present.

### 1.5.7 Summary

Respiratory control is a balanced system with numerous components. Sudden infant death syndrome and neonatal apnoea may both be manifestations of imbalance within these systems of homeostatic control.

The mechanisms by which the established risk factors for SIDS impart vulnerability remain unclear. In this thesis I examine the effect of certain risk factors on the hypercarbic and hypoxic responses in the neonatal period. By exploring the physiological effects of these risk factors it may be possible to suggest mechanisms by which the vulnerability to SIDS occurs.

In the preterm population disturbance of respiratory control is common, but incompletely understood. The ventilatory response to hypercarbia is a critical homeostatic mechanism, and I will explore whether this is compromised in preterm infants, and whether the pharmacological therapy for apnoea, caffeine, has an effect on this ventilatory response.

In exploring the pathogenesis of apnoea in the neonatal population I will consider the potential role of gastro-oesophageal reflux. Both apnoea and gastro-oesophageal reflux are common in the neonatal intensive care population, and although an association has been suspected this has been inconsistently demonstrated, possibly as a result of historic limitations in investigation modalities. Therefore in this thesis I will evaluate the different methods of investigation for gastro-oesophageal reflux in the neonatal population, and use multichannel intraluminal impedance in combination with pH studies to critically evaluate any association between gastro-oesophageal reflux and apnoea.

## 1.6 Hypotheses:

- In term infants, maternal substance misuse and maternal smoking will dampen the ventilatory response to hypoxia and hypercarbia and this will be more marked in the prone position.
- Carbon dioxide sensitivity will increase with postnatal age and caffeine therapy and will be associated with a reduction in apnoea
- A proportion of apnoeas will be associated with gastro-oesophageal reflux events

## 1.7 Aims

- To measure the ventilatory response to hypoxia and hypercarbia using the steady state technique in healthy term infants of mothers who have smoked in pregnancy, misused substances in pregnancy, or neither smoked nor misused substances in both the prone and supine sleeping position

- In prematurely born infants measure the ventilatory response to hypercarbia and assess the effect of gestational and postnatal age and caffeine therapy

- Evaluate variation in practice in the investigation and management of gastro-oesophageal reflux disease on neonatal units in the United Kingdom

- Evaluate the use of pH/MII in the detection of reflux events in infants on the neonatal unit, and compare the results with that obtained by upper gastro-intestinal contrast study

- Using pH and multichannel intraluminal impedance studies examine the association between gastro-oesophageal reflux and apnoea in infants with suspected GOR

# Chapter 2 : Methods

## 2.1 Protocols

A series of protocols were carried out:

### 2.1.1 The effects of smoking and substance misuse in pregnancy and infant sleeping position on respiratory control in infants

To determine ventilatory responses to hypercarbia: measurement of respiratory airflow and $CO_2$ levels were carried out for a period of five minutes at each of three levels of inspired $CO_2$ (0%, 2% and 4% $CO_2$ in air). The outcomes assessed were:

- The increase in minute ventilation in response to increased concentration of inspired $CO_2$
- The increase in mean inspiratory flow in response to increased concentration of inspired $CO_2$
- The time constant of the increase in minute volume in response to inspiring 4% $CO_2$

To determine the ventilatory response to hypoxia, the same measurements as described above including oxygen saturations were performed during a five minute period breathing air (21% oxygen) followed by a five minute period breathing hypoxic gas (15% oxygen balanced with nitrogen). The outcomes assessed were:

- Increase in minute ventilation in response to hypoxia
- The time to maximum minute volume in response to hypoxia
- The magnitude of decline in minute ventilation during prolonged hypoxia
- The rate of decline in minute ventilation during prolonged hypoxia
- The minimum oxygen saturation during hypoxic challenge

### 2.1.2 The effect of prematurity on central chemoreceptor sensitivity and respiratory pattern in the newborn

To evaluate the effect of prematurity and caffeine therapy on respiratory control, infants born at less than 34 weeks gestation who were not requiring respiratory support and had not received caffeine therapy were studied. The ventilatory response to hypercarbia was measured. To determine ventilatory responses to hypercarbia: measurement of respiratory airflow and oxygen saturation were carried out for a period of five minutes at each of three levels of inspired $CO_2$ (0%, 2% and 4% $CO_2$ in air). Infants were studied weekly until neonatal unit discharge. At each study, it was noted whether they were receiving caffeine therapy. The outcome measured was:

- The increase in minute volume in response to increased concentration of inspired $CO_2$

### 2.1.3 The detection, diagnosis and treatment of gastro-oesophageal reflux disease and its association with apnoea

In these studies I aimed to evaluate current investigation and management of GORD on neonatal units in the United Kingdom, determined whether investigations capable of detecting non-acid reflux might improve detection of GORD and if there was an association between reflux episodes and apnoea.

i. To evaluate current practice a survey was undertaken of all neonatal units in the United Kingdom regarding investigation and management of suspected gastro-oesophageal reflux disease and medication used.

ii. To evaluate the role of non-acid reflux in GORD and use of multichannel intraluminal impedance in the detection of GORD, combined multichannel intraluminal and impedance and pH equipment was used to investigate infants on the neonatal unit suspected of having GORD.

iii. A comparison was made of the results of combined multichannel intraluminal impedance and pH studies and upper gastro-intestinal contrast studies in infants investigated for suspected gastro-oesophageal reflux disease.

iv. To determine if gastro-oesophageal reflux was related to apnoea, combined multichannel intraluminal impedance and pH equipment with synchronised polysomnography was employed. Acid and non-acid reflux, respiratory air-flow, chest and abdominal movements, electrocardiography, oxygen saturation and activity were monitored continuously.

## 2.2 Subjects

Subjects were recruited from the Neonatal Intensive Care Unit or postnatal ward at King's College Hospital NHS Foundation Trust, Denmark Hill, London between September 2012 and September 2015. Eligible participants were identified and the parents asked if they were willing for their infant to participate in a study. All parents approached were provided with written participant information and given a minimum of 24 hours to consider before providing written informed consent. The studies were approved by the London Bromley Research Ethics Committee ("the effect of smoking and substance misuse in pregnancy and infant sleeping position on respiratory control in infants study" and "the effect of prematurity on central chemoreceptor sensitivity and respiratory pattern in the newborn study" and the London Riverside Research Ethics Committee ("the gastro-oesophageal reflux and apnoea study").

## 2.3 Ventilatory responses to hypercarbia and hypoxia

### 2.3.1 Equipment

The test gas was delivered using an open circuit system via a nasal mask and pneumotachograph. The pneumotachograph (Mercury F10L, G M Instruments, Kilwinning, Scotland) has a dead space 0.8 ml and resistance 0.86 $mmH_2O/L/min$ (manufacturer's data) and was connected to a soft latex nasal mask (Neomask, Draeger, Germany) using a snugly fitting connector. The mask was placed over the infant's nose. A seal was achieved by gentle pressure, and confirmed by assessing leak (discrepancy between inspired and expired tidal volumes) in real time from the computer display. The distal end of the pneumotachograph was connected to the common port of a two-way non-rebreathing valve which separated inspired from expired gas and ensured that the controlled mixture of gases was inspired by the infant, whilst allowing expiration to the outside air. A constant flow of medical air was delivered to the inspiratory port of the valve via wide bore (20 mm), low resistance tubing eliminating any dead space. The inspired air could be enriched with a variable concentration of $CO_2$ from a cylinder. The pneumotachograph was connected to a differential pressure transducer-amplifier system (Gould model 13-4615-70, Cleveland OH, USA) generating an analogue signal proportional to airflow. A capnograph ($CO_2$SMO capnograph; (Respironics UK, Chichester, UK) sampled gas continuously from the nasal mask through a fine bore catheter at a rate of 180ml/minute. The carbon dioxide content of the sampled gas was determined by infrared spectroscopy and an analogue signal proportional to the $CO_2$ concentration generated. Oxygen saturation was measured using a pulse oximeter (Massimo rainbow SET Pulse Oximetry) attached to the foot of the infant.

Respiratory flow and gas concentration were acquired and displayed in real time on a PC computer running Spectra software (Grove Medical, London, UK) with 100 Hz analogue to digital sampling (PCI-MIO-16XE-50, National Instruments, Austin TX, USA).

### 2.3.2 Ventilatory response to hypercarbia

Following a five-minute period breathing medical air, the hypercarbic challenge was delivered through the equipment described above. $CO_2$ was delivered from a cylinder of $CO_2$ 20% balanced with medical air (BOC), via a low-flow meter. Continuous capnograph read out allowed adjustment to deliver 2% or 4% $CO_2$.

### 2.3.3 Ventilatory response to Hypoxia

Following a five-minute period breathing medical air, the hypoxic gas comprising 15% Oxygen balanced with nitrogen was delivered to the infant through the equipment described above during the hypoxic challenge. Continuous saturation monitoring was undertaken in addition to the measurements above.

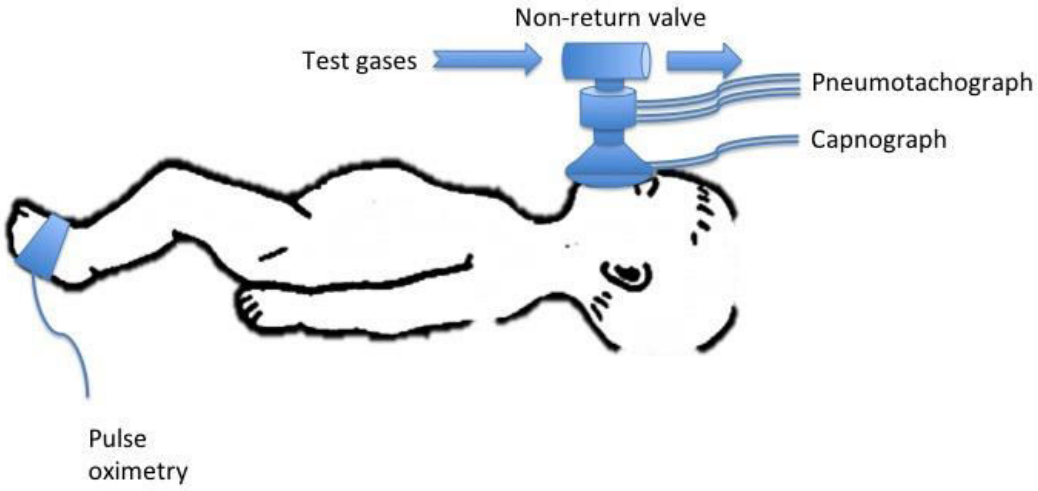


Figure 7: Schematic of the ventilatory challenge equipment

## 2.4 Equipment calibration

The pneumotachograph and differential pressure transducer were calibrated prior to each study using a rotameter. Linearity of the pneumotachograph was assessed by plotting the digital output from the acquisition software against air flow -10 to 10 L/min. A linear relationship between system output (analogue to digital units (AD)) and flow was observed up to 10 L/min in both directions of flow through the pneumotachograph. (Figure 8)

The capnograph was calibrated prior to each study using certified calibration gas (5% $CO_2$ /95%) (BOC gases, United Kingdom). (Figure 9) The linearity of the capnograph response was assessed by plotting the digital output from the acquisition system software against three different concentrations of $CO_2$; 0% (medical air), 5% $CO_2$ /95% air and 7.5% $CO_2$ /92.5% air (BOC gases, United Kingdom). The calibration gases were certified to have an error of less than 5% of nominal concentration i.e ±0.25% for the 5% $CO_2$ and ±0.375% for the 7.5% $CO_2$. A linear relationship was observed between capnograph output and $CO_2$ concentration (Figure 9)

Data regarding accuracy and linearity of response of the Massimo rainbow SET Pulse Oximeter was obtained from the product literature. Accuracy to within 2% of stable arterial blood gas readings was demonstrated in healthy adult volunteers across a range of oxygen saturations between 70 and 100%.(Massimo, 2014)(Figure 10)

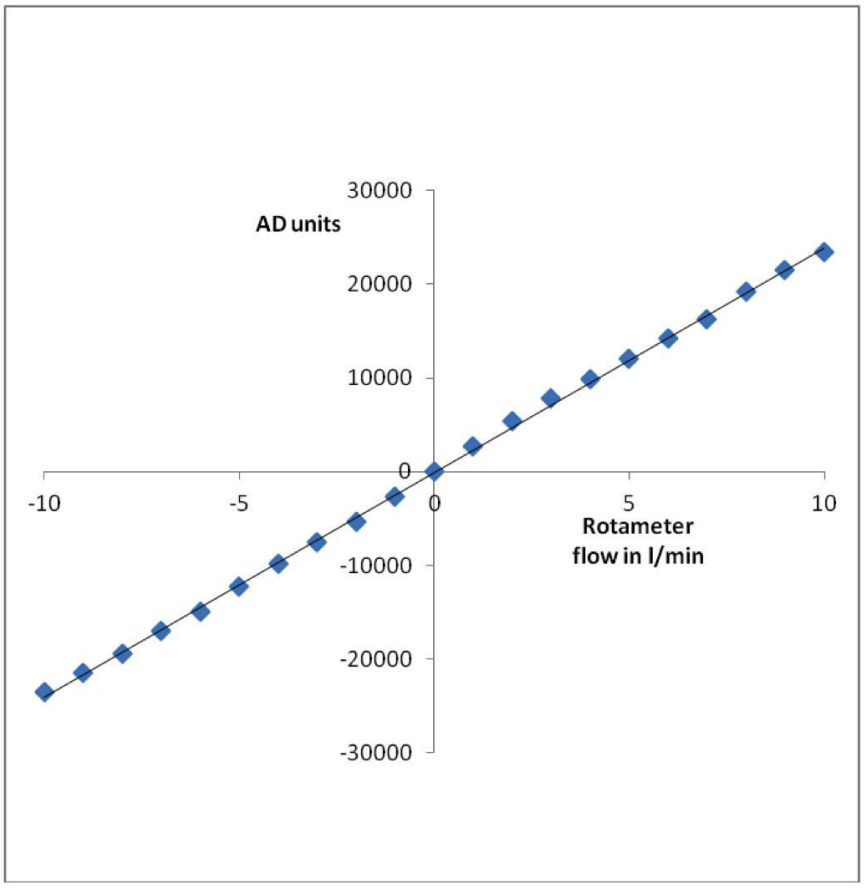


Figure 8: Linearity of pneumotachograph and associated pressure transducer

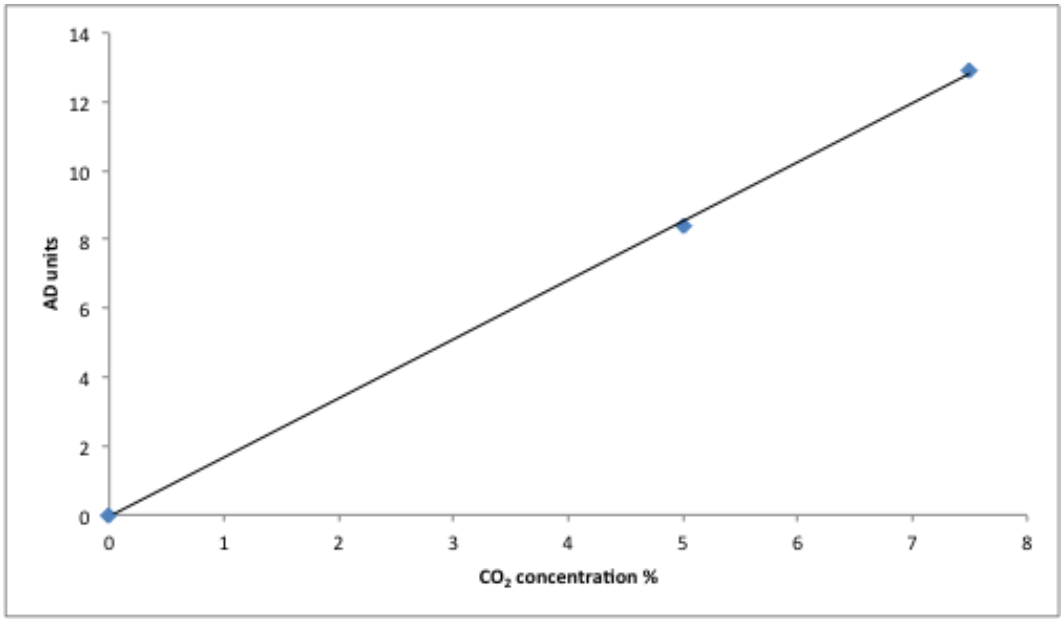


Figure 9: Linearity of capnograph response

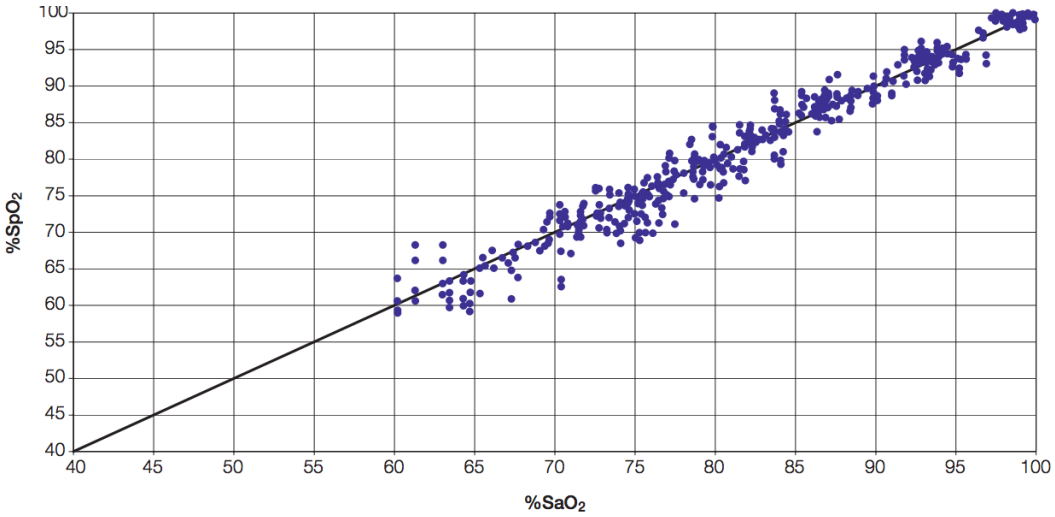


Figure 10: Plot of 636 data pairs of $SpO_2$ versus $SaO_2$ in 17 healthy adult volunteers in the 60% to 100% $sao_2$ range during normothermix no motion conditions(Massimo, 2014).

## 2.5 Procedure

Assessment of the ventilatory responses to hypercarbia and hypoxia were performed early in the morning while the infants were asleep. In those infants receiving enteral feeds the measurements were made after a feed. Measurements were made during quiet sleep (State 1) as defined by the Prechtl criteria (Prechtl, 1974):

State I: eyes closed, regular respiration, no movements.

State 2: eyes closed, irregular respiration, small movements.

State 3: eyes open, no movements.

State 4: eyes open, gross movements.

State 5: crying (vocalisation).

If the infant aroused the measurement was abandoned and restarted once the infant had returned to quiet sleep.

### 2.5.1 Hypercapnic challenge

The hypercapnic challenge was delivered using the equipment as described above. Ventilation was measured during exposure to three levels of $CO_2$ (0% (baseline), 2% and 4%). These levels were chosen to allow measurement of changes in ventilation elicited by linear increases in inspired $CO_2$, without generating significant arousal, and are in line with previous studies.(Rigatto et al., 1975, Ali et al., 2014, Saiki et al., 2014, Smith et al., 2010) Each mixture of $CO_2$ was individually titrated and a stable inspiratory $CO_2$ concentration achieved within the delivery tubing as assessed using the continuous measure of gas concentration from the capnograph. The infant breathed the air/$CO_2$ mixture for at least five minutes to allow ventilation and $ETCO_2$ to reach steady state as assessed from the real-time display. The order of test gases was randomised for each infant.

### 2.5.2 Hypoxic challenge

The hypoxic challenge was delivered using the open circuit equipment as described above. Baseline ventilation was measured while breathing medical air from a cylinder for five minutes prior to switching to a premixed gas containing 15% oxygen in nitrogen delivered from a cylinder (BOC Gases, UK. Exposure to the hypoxic gas was maintained for a further 5 minutes. The hypoxic challenge was maintained for five minutes. The test was terminated if transcutaneous oxygen saturation fell below 85%. The last minute of tidal breathing prior to switching to the hypoxic gas mix was used as a baseline value.

## 2.6 Assessment of exposure to smoking and substance misuse

### 2.6.1 Urinary cotinine levels

Urine samples were collected in universal specimen containers, in the postnatal period after obtaining consent from the parents, aliquoted and then stored at -20°C until assayed. The cotinine reagent was supplied by Siemens Healthcare Diagnostics Ltd, Newton House, Sir William Siemens Square, Frimley, Camberley, Surrey GU16 8QD. The analysis was performed on the Siemens Immulite 2000 analyser. The assay was a solid phase competitive chemiluminescence immunoassay for the measurement of Cotinine in human serum or urine. It utilises one rabbit polyclonal antibody, which is bound to a bead and cotinine which is conjugated to alkaline phosphatase. The reagents were placed on a Siemens Immulite 2000.

The patient sample and reagent were incubated together with the coated bead for 30 minutes. During this time, cotinine in the sample and cotinine which was conjugated to alkaline phosphatase in the reagent compete for binding sites on the beads. Unbound patient sample and excess reagent were then removed by centrifugal washes. Finally, chemiluminescent substrate was added to the reaction tube containing the bead and the signal was generated which was in proportion to the bound enzyme and inversely proportional to the amount of cotinine present in the sample.(Viapath, 2015a) The Cotinine assay is linear up to 500 μg/L. Samples with Cotinine concentrations greater than 500 μg/L were diluted with multi-diluent 2 supplied by Siemens and re-assayed. The sensitivity of the cotinine assay was 5.0 μg/L

### 2.6.2 Substance misuse

In the immediate postpartum period, urine samples were taken from the mothers. Urine samples were aliquoted and then stored at -20°C until assayed.

Urine samples were tested using assays on the CEDIA principle (Cloned Enzyme Donor ImmunoAssay), for cannabinoids, opiates, amphetamines, methadone, cocaine and benzodiazepines.

CEDIA technology is based on beta-galactosidase that has been synthesised in two inactive fragments that will spontaneously re-associate to form active enzyme. The sample and reagent 1 were mixed. Reagent 1 consists of one fragment of beta-galactosidase that has the drug being assayed conjugated to it, and antibodies to that drug.

After incubation, the antibody was competitively bound to either the free drug or the drug conjugated to the beta-galactosidase fragment. Reagent 2 was added, containing the second fragment of the beta-galactosidase and the substrate. The two parts of the beta-galactosidase spontaneously reassociate to form active enzyme only when there is no antibody bound to the fragment from reagent 1. The active enzyme cleaves the substrate, forming a coloured product that can be measured spectrophotometrically. The rate of formation of this product is dependent on the amount of active enzyme, which is dependent on the amount of free drug in the sample that is able to bind to antibodies. (Viapath, 2015b)

The cut-off concentrations used to determine positivity were those recommended by the manufacturer (cannabinoids 20 lg/l, opiates 300 lg/l, amphetamines 1000 lg/l, methadone 300 lg/l, cocaine 1000 lg/l and benzodiazepines 300 lg/l).

## 2.7 Polysomnography

Polysomnography was performed on the neonatal intensive care or special care baby unit.

### 2.7.1 Equipment

Polysomnography was performed using the commercially available Alice 4 sleep study unit (Profile Vio-systems, Bognor Regis, UK) using the Alice 5 firmware upgrade to allow data export and integration of synchronisation signals which could not be performed with the original Alice 4 software. Integration of a synchronisation signal at the start and end of recording ensured that when evaluating pH/MII traces and polysomnography traces the time base for each recording was synchronised, and any discrepancy in internal computer clock was corrected for.

Abdominal and thoracic movements were measured using stretch sensitive piezo-electric respiratory bands. Oral and nasal airflow was detected using a thermistor which utilised temperature differentials generated by airflow movement to indicate respiratory airflow. A single channel lead 2 electrocardiogram was recorded using single use bipolar electrodes to monitor heart rate. Two activity meters (Profile Vio-systems, Bognor Regis, UK) were attached to the arm and leg to record infant limb movements to assess for movement artefact and assist with sleep staging. Oxygen saturation was continuously monitored using a pulse oximeter (Massimo rainbow SET Pulse Oximetry) and was incorporated into the Alice sleep system using an auxiliary input.

The Alice sleep system was connected to a PC which was used to display recordings in real time and store collected data. A custom built synchronisation box provided a synchronisation signal to an auxiliary input to the Alice sleep system, and the multichannel intraluminal impedance system when required.

### 2.7.2 Analysis

#### 2.7.2.1 Apnoeas

Apnoeas were defined as cessation of respiratory airflow for a minimum of five seconds. For each apnoea associated changes in heart rate and oxygen saturations were recorded. The apnoeas were classified into central, obstructive and mixed apnoeas according to the following criteria:(Butcher-Puech et al., 1985)

Central apnoea was defined as a cessation of respiratory airflow and thoraco-abdominal movements for at least five seconds. (Figure 11)

Obstructive apnoea was defined as a cessation of respiratory airflow with persistence of thoraco-abdominal movements for at least five seconds. (Figure 12)

Mixed apnoea was defined as a central apnoea with at least one respiratory effort without successful generation of respiratory airflow. (Figure 13)

Periodic breathing was defined as at least three pauses of more than three seconds, interspersed by periods of breathing not longer than 20 seconds. When exploring the association between gastro-oesophageal reflux and apnoea, those apnoeas which occurred within a period of periodic breathing were not included in the analysis.

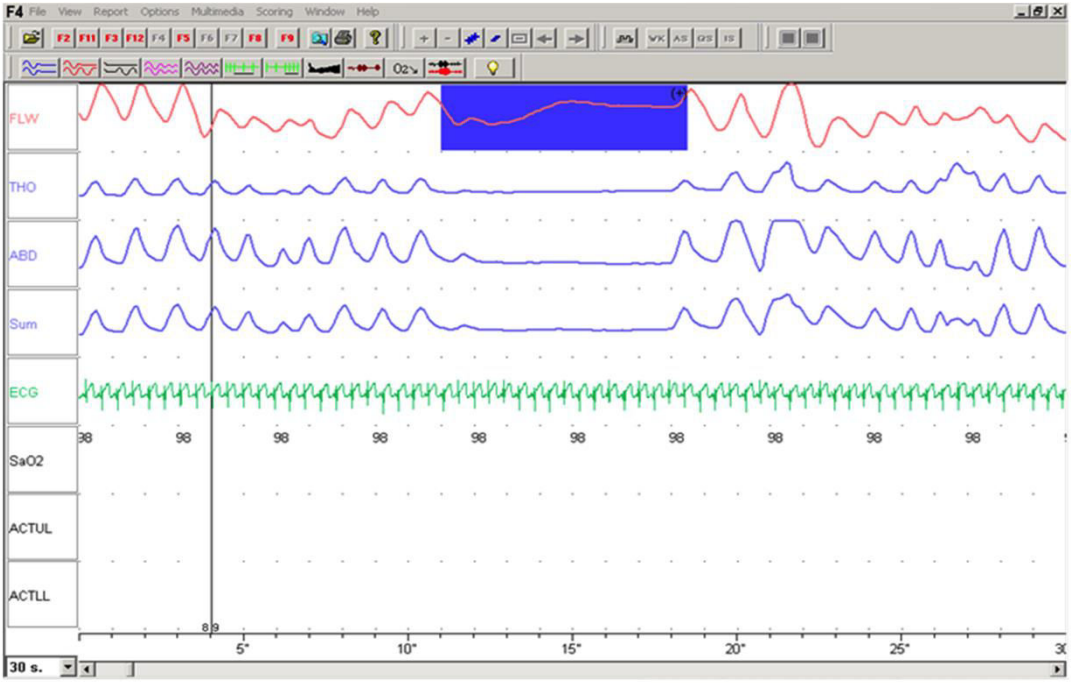


Figure 11: Polysomnograph showing a central apnoea.

FLW = respiratory air flow; THO = thoracic band; ABD = abdominal band; SUM = sum of abdominal and thoracic band stretch; ECG = single lead electrocardiogram; $SaO_2$ = transcutaneous oxygen saturation; ACTUL = upper limb movement sensor; ACTLL = lower limb movement sensor

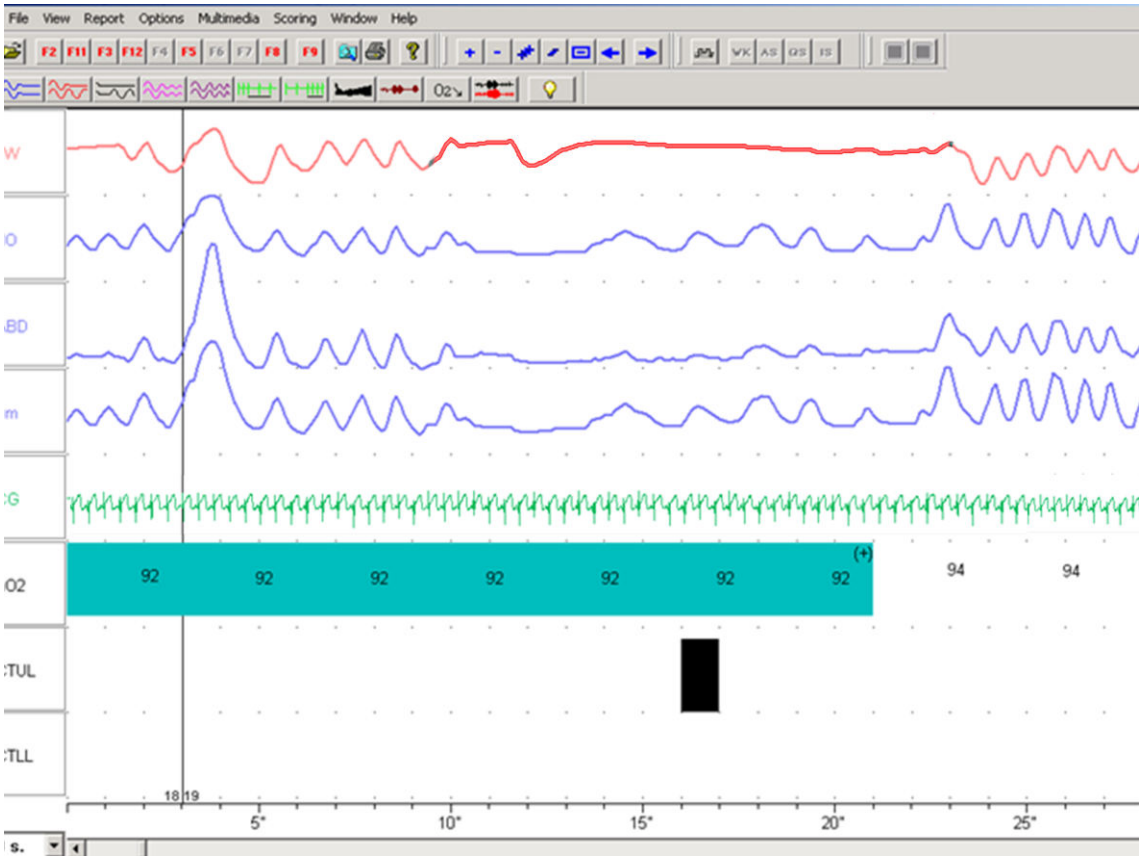


Figure 12: Polysomnograph showing an obstructive apnoea

FLW = respiratory air flow; THO = thoracic band; ABD = abdominal band; SUM = sum of abdominal and thoracic band stretch; ECG = single lead electrocardiogram; $SaO_2$ = transcutaneous oxygen saturation; ACTUL = upper limb movement sensor; ACTLL = lower limb movement sensor

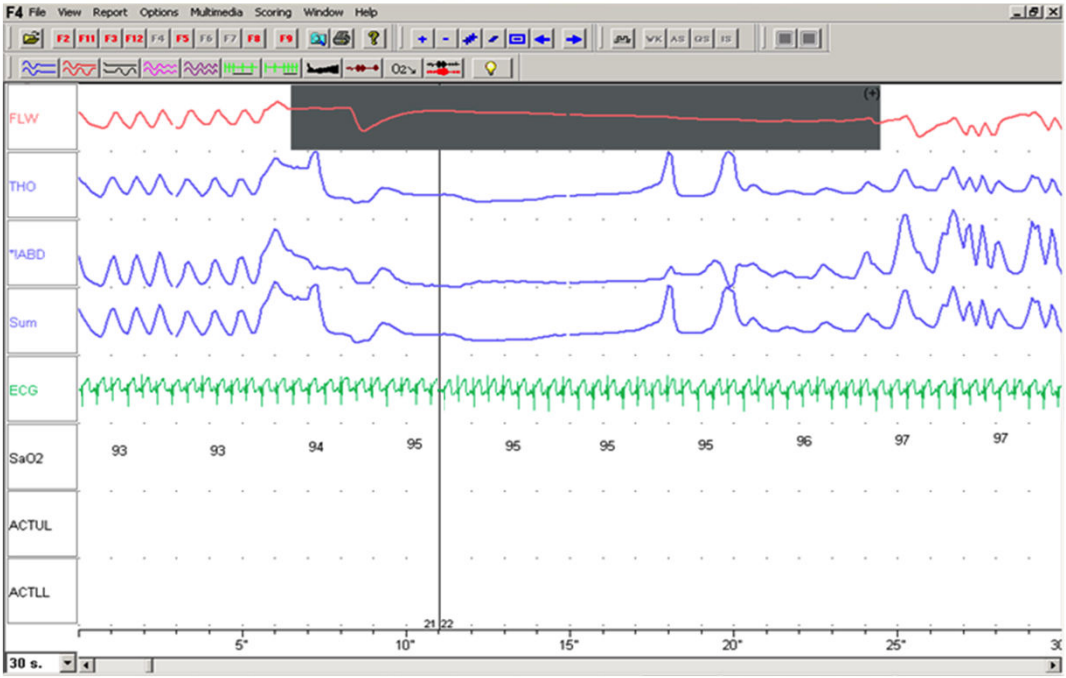


Figure 13: Polysomnograph showing a mixed apnoea

An initial central apnoea is followed by at least one respiratory effort without generating air flow. The combination of central and obstructive apnoea is defined as a mixed apnoea. FLW = respiratory air flow; THO = thoracic band; ABD = abdominal band; SUM = sum of abdominal and thoracic band stretch; ECG = single lead electrocardiogram; $SaO_2$ = transcutaneous oxygen saturation; ACTUL = upper limb movement sensor; ACTLL = lower limb movement sensor

## 2.8 Combined pH and Multichannel intraluminal impedance monitoring

### 2.8.1 Equipment

Infants with suspected GORD were assessed as part of their routine clinical care. Each infant underwent a minimum of 20 hours of continuous oesophageal pH and MII assessment. A single use combined pH/MII probe (Zin51 probe, Sandhill Scientific, Highland Ranch, Colorado, USA) was used which incorporated seven impedance bands allowing measurement of impedance across six channels each with a width of 1.5cm. Between the two most distal bands was an antimony pH sensor. Prior to each study, the pH sensor was calibrated with pH buffer solutions of pH 4.0 and pH 7.0 and an automated impedance check was performed by the Zephyr Sleuth system (Sandhill Scientific). The infant's length was measured and oesophageal length estimated according to Strobel's formula for infants over 40cm (Strobel et al., 1979) and by a nomogram for those under 40cm (Omari et al., 1999). The probe was inserted through a nostril and secured at the required position. A chest radiograph was then obtained to determine if the pH sensor was appropriately positioned between the seventh and ninth thoracic vertebra.(Di Fiore et al., 2009) The position of the probe at the nares was reassessed following completion of the study to ensure the probe had not been displaced.

Following confirmation of probe position, recording was commenced. The Zephyr Sleuth system (Sandhill Scientific) continuously recorded impedance and pH data with a sampling frequency of 50 hertz. Analysis of the traces produced was performed using Bioview Analysis software (Sandhill Scientific) and by manual review of the traces.

### 2.8.2 Equipment calibration

Prior to each study the combined pH/MII probe was calibrated. A two point calibration was performed on the pH probe using buffer solutions of pH 4.01 and pH 7.01 (Sandhill Scientific, Highland Ranch, Colorado, USA). An automated impedance check was carried out prior to each study using the Sleuth Zephyr software (Sandhill Scientific, Highland Ranch, Colorado,

USA). Linearity of response of the pH probe was confirmed using buffer solutions of known pH (1.07, 4.01, 7.01) (Figure 7). Linearity of impedance response was determined by bridging a channel with resistors of known impedance (Figure 8).

### 2.8.3 Reproducibility

To determine the reproducibility of MII scoring, ten randomly selected studies were independently scored by two researchers. The number of impedance derived reflux events were compared between the two observers by calculating the intra-class correlation coefficient, assuming a two-way mixed effect model. The intra-class correlation coefficient was 0.985 (95% CI, 0.899-0.997).

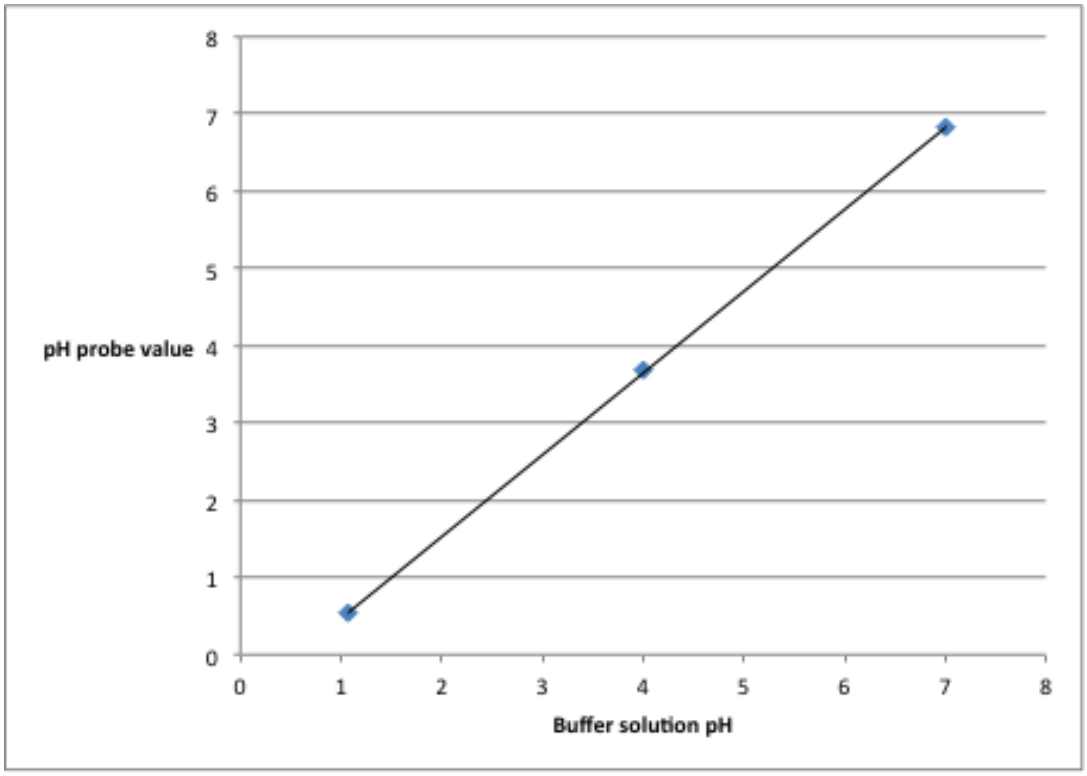


Figure 14: Linearity of the pH probe

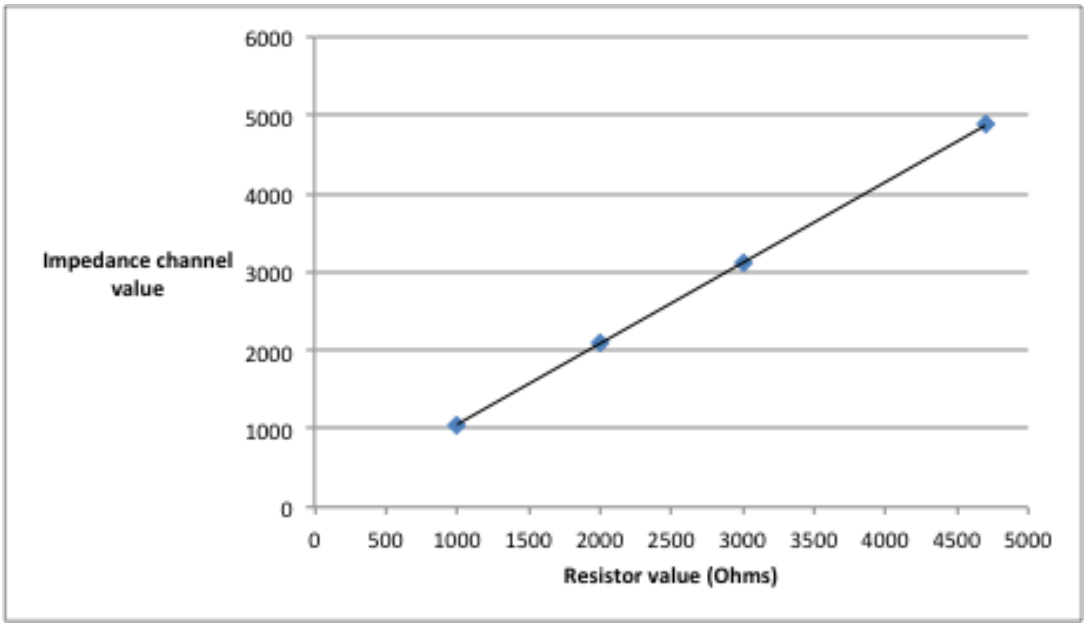


Figure 15: Linearity of the impedance probe

### 2.8.4 Analysis

A pH probe reflux event was defined as a drop in oesophageal pH to less than four for more than five seconds.(Woodley and Mousa, 2006) The total number of pH events per 24 hours was calculated. The duration of the reflux event and the acid clearance time (ACT) (the time from the pH dropping below four to rising above four) were determined. The mean ACT (the total duration with pH <4 divided by the number of acid reflux events) was calculated and the maximum ACT identified. The acid index was the total time spent with the oesophageal pH less than four as a percentage of the total study time. GORD was diagnosed if the acid index was greater than 11.7% (Vandenplas et al., 1991).

MII reflux events were defined as a drop in impedance to less than 50% of the baseline at the most distal channel which moved retrogradely across at least two channels. These were further classified as either acid (pH <4)(Figure 16), weakly acid (4> pH <7)(Figure 17) or alkali (pH >7). A pH only event was defined as a drop in oesophageal pH to below 4 without associated changes in impedance sufficient to diagnose an MII reflux event. (Figure 18) The duration of a reflux event, the bolus clearance time (BCT), was the time from the drop in impedance to less than 50% of baseline to the time that the impedance rose above that threshold. The mean BCT was calculated, and maximum BCT observed. GORD was diagnosed if the number of impedance detected events was greater than 79, which was the $90^{th}$ centile in a study of forty-six healthy infants investigated for possible reflux related symptoms, who had no symptom association and an acid index of less than the median acid index value derived from previous studies.(Mousa et al., 2014, Vandenplas et al., 2009) This was roughly equivalent to the $75^{th}$ centile in a study of 20 asymptomatic, prematurely born infants.(Lopez-Alonso et al., 2006)

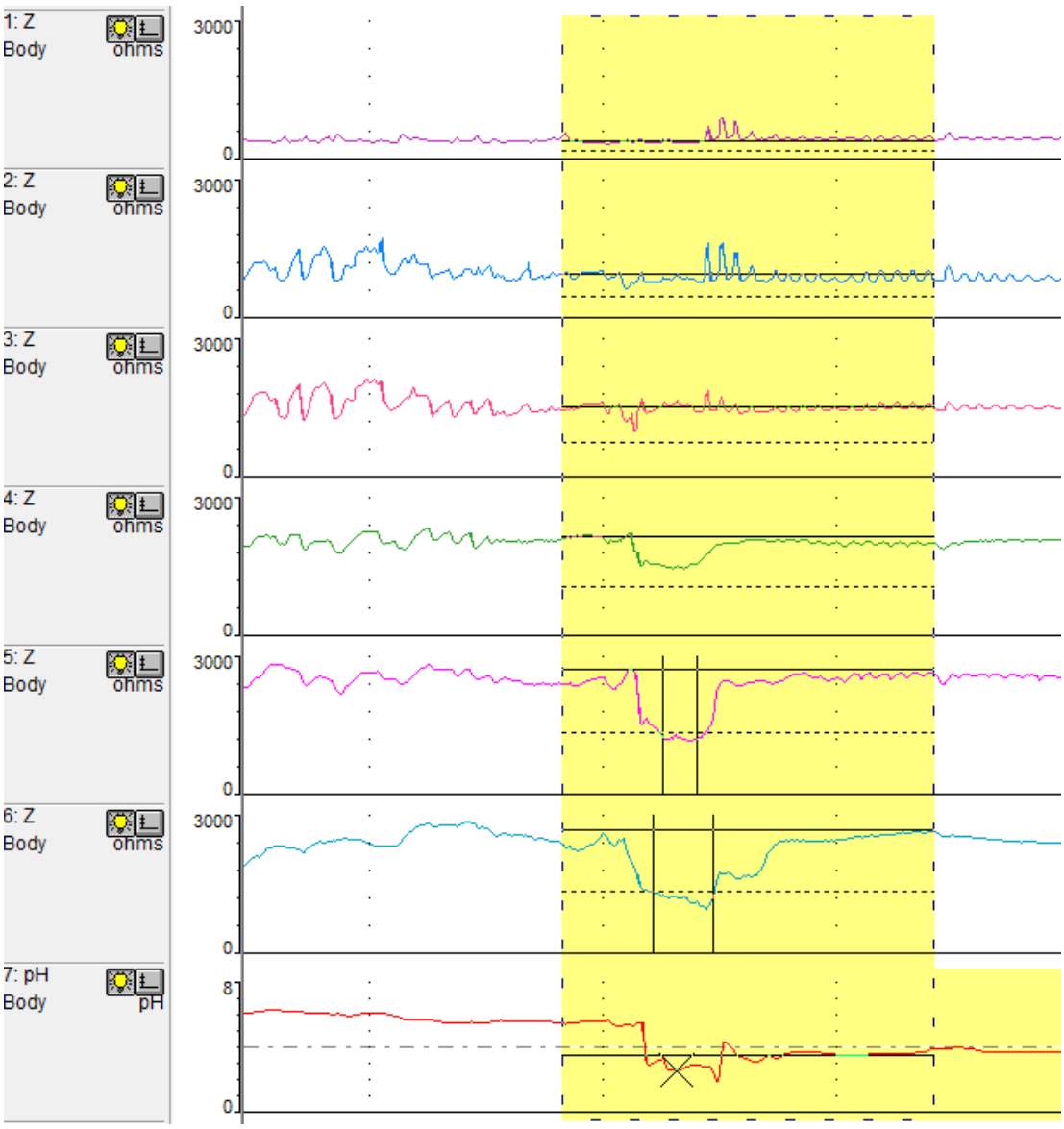


Figure 16: Screen shot of MII/pH trace showing an acid MII event.

There is a drop in impedance of more than 50% of baseline in the most distal impedance channel (6) moving retrogradely cross one further channel (5). This coincides in a drop in pH (channel 7) to below pH 4

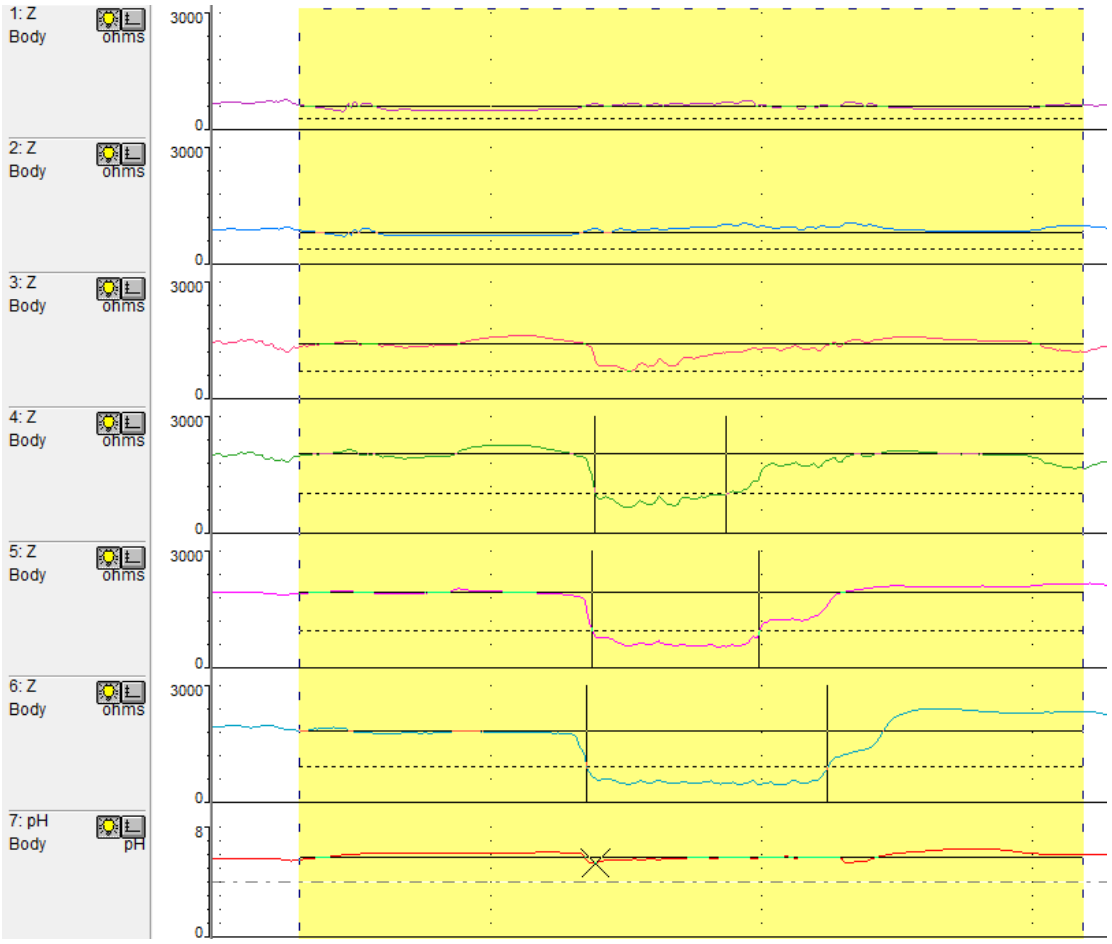


Figure 17: Screen shot of MII/pH trace showing a non-acid MII event.

There is a drop in impedance of more than 50% of baseline in the most distal impedance channel (6) moving retrogradely across two further channels (5 & 4). This event is not associated with any drop in pH (channel 7) which remains above pH 4

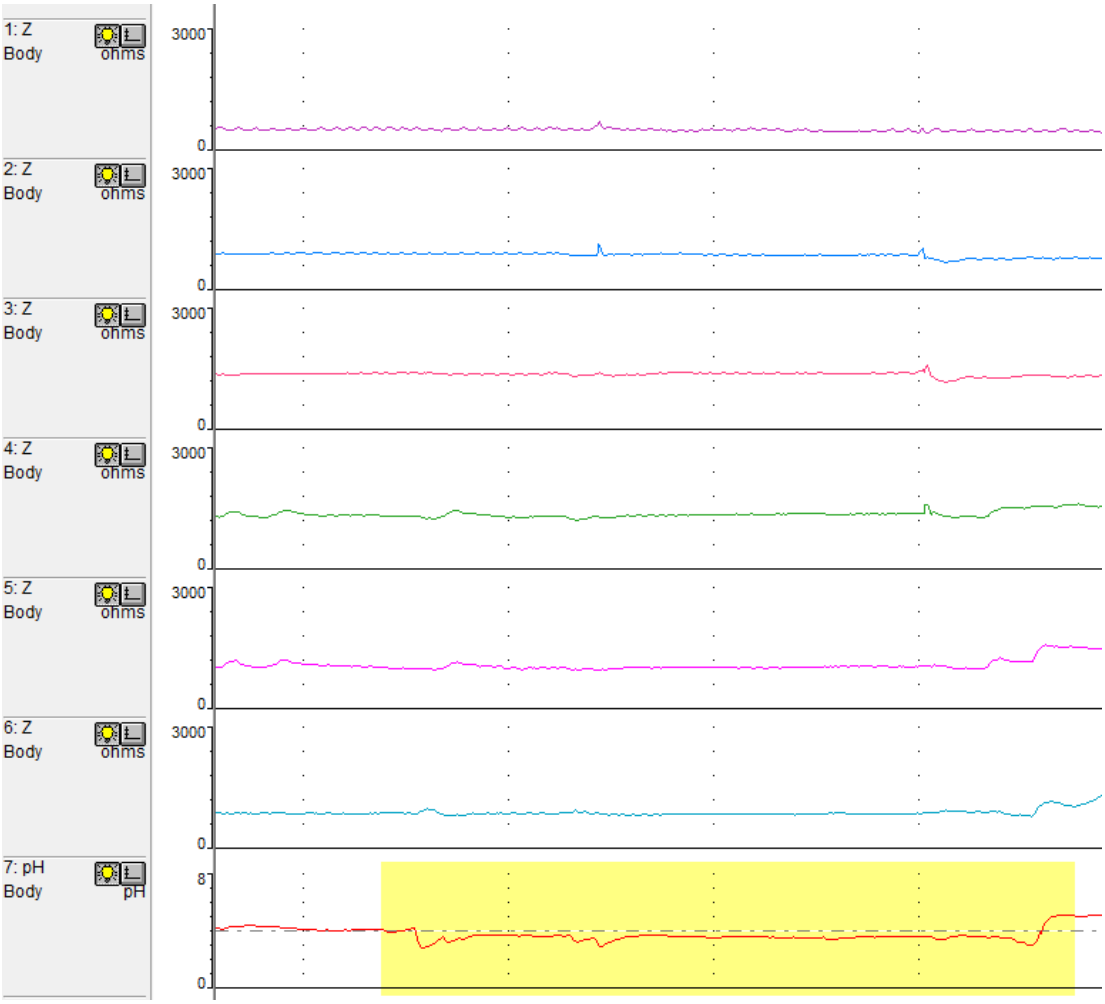


Figure 18: Screen shot of MII/pH trace showing a pH only event.

There is no drop in impedance apparent but a drop in pH (Channel 7) to less than 4

### 2.8.5 Baseline oesophageal impedance

Baseline impedance values were calculated for the most distal channel using the algorithm developed and validated by Loots et al. to determine the baseline intraluminal impedance when the effect of gas and liquid boluses is excluded.(Loots et al., 2011) This algorithm automatically excluded periods of bolus and gas transit from the impedance traces to evaluate the resting impedance of the distal oesophagus.

Each channel trace was divided into 10 minute sections which were then subdivided into 10 second epochs. The minimum value for each 10 second epoch was identified. The 60 data points collected for the 10 minute section were then filtered, those lying more than one standard deviation from the section mean were excluded as they were likely to be due to periods of bolus transit.(Loots et al., 2011) The mean for the remaining points in the 10 minute period was calculated and the median for the whole study determined.

# Chapter 3 : The effect of maternal smoking and substance misuse and infant sleeping position on the ventilatory response to hypercarbia in the newborn period.

Despite the significant reduction in the number of cases of Sudden Infant Death Syndrome following the 'Back to Sleep' campaign in which parents were advised to avoid prone sleeping, there were over two thousand SIDS cases in the USA in 2012.(Mathews and MacDorman, 2013) Following the 'Back to sleep' campaign other risk factors, particularly maternal smoking which was present in 86% of SIDS cases in England and Wales, and maternal substance misuse, are present in a greater proportion of SIDS cases.(Blair et al., 2006)

Twelve percent of pregnant women in the UK smoked throughout their pregnancy.(McAndrew et al., 2010) Anonymous analysis of all positive urine pregnancy test samples presented to clinics in south London over a six month period demonstrated that 15.6% of the mothers were taking at least one substance of misuse.(Sherwood et al., 1999)

Maternal smoking during pregnancy has been reported to increase SIDS risk, and a meta-analysis of 35 case-control studies estimated an approximately two-fold increase in risk of SIDS in infants of mothers who smoked in pregnancy compared to controls (OR = 2.25, 95% CI = 2.03–2.50).(Zhang and Wang, 2013) Furthermore the risk was dose dependent, with heavier smoking mothers having infants at greater risk. The increased risk of SIDS in infants of substance abusing mothers (ISAMs) compared to controls has been reported from four to eight fold.(Kandall et al., 1993, Ward et al., 1990)

The effect of combinations of risk factors may be more than additive. Oyen et al. in the Nordic Epidemiological SIDS study found an odds ratio (OR) of 4.9 in infants of mothers who smoked in pregnancy compared to controls sleeping supine, however the OR increased to 55 when the combination of maternal smoking in pregnancy and prone sleeping were present.(Oyen et al., 1997) The combined risk of prone sleeping and maternal substance misuse is unreported.
Risk factors for SIDS may increase vulnerability by impairing infant respiratory control, and reducing the infant's ability to respond to an exogenous stressor. Therefore exposure in-utero to maternal smoking or maternal substance misuse may be associated with an impaired ventilatory response to hypercarbia, and this impairment may be more marked when sleeping in the prone compared to the supine position.

In this study, I have tested the hypothesis that the ventilatory response to hypercarbia would be more damped in infants of smoking and/or substance misusing mothers compared to that of controls, particularly in the prone compared to supine position.

## 3.1 Methods

Infants were eligible for inclusion in the study if they were born at 36 weeks of gestation or greater at King's College Hospital NHS Foundation Trust. They were studied prior to discharge from the maternity unit. Exclusion criteria were major congenital abnormalities, respiratory disease or sepsis. Approval for the study was obtained from the London – Bromley Research Ethics Committee. Parents gave informed written consent for their infant to participate.

### 3.1.1 Protocol

Infants were studied prior to maternity unit/neonatal unit discharge at between 36 and 42 weeks corrected gestational age. After a feed, the infant was placed in either the prone or supine sleeping position, the other position being studied afterwards on the same day. The order in which the positions were studied was randomised between infants. Measurements were made while the infant was in quiet sleep. Sleep state was determined by observation of the behavioural state.(Prechtl, 1974) An infant was defined as being in quiet sleep when their eyes were closed, with no body or eye movement, no vocalisation and their respiratory rate was regular. If arousal occurred the measurement was abandoned and recommenced once the infant returned to quiet sleep. The hypercarbic challenge was delivered via a nasal mask and open circuit system as described in Chapter 2.

The data from each ventilatory challenge were recorded digitally using the Spectra software for later review and analysis. Respiratory rate, inspired tidal volume, expired tidal volume, inspiratory time, expiratory time, heart rate, oxygen saturations and end-tidal $CO_2$ level were measured breath by breath.

Respiratory rate was determined from the flow signal. Tidal volume was determined by digital integration of the flow signal by the acquisition software. Mean inspiratory flow (MIF) was

calculated by dividing the tidal volume by the inspiratory time. Minute ventilation was calculated from the infant's respiratory rate and tidal volume and related to the infant's weight, as maternal smoking and substance misuse are known to affect birth weight.(Braun et al., 2010) Inspired and expired gases were sampled continuously using a small cannula inserted through the nasal mask.

The final minute of tidal breathing was used as the baseline value. Infants were switched from breathing medical air to a mixture either of 2% or 4% $CO_2$ determined by the continuous capnograph readout. The order of exposure to different $CO_2$ levels was randomised. The infant was exposed to each test gas for five minutes, with a minimum five-minute washout period breathing air between the test gas exposures.

The mean value for minute volume and MIF were calculated over the fifth minute of gas exposure during quiet sleep and were plotted against the inspired concentration of $CO_2$. The gradient of the line of best fit for each variable was calculated as a marker of carbon dioxide sensitivity.

The ventilatory response to 4% $CO_2$ was modelled as a response to a step input of a first-order, linear time-invariant system, and the rate of response described using the time constant. The time constant reflects the time taken for a system to reach steady state if the response was linear and continued at the initial rate of change. As the rate of change decreases, the time constant reflects the time taken to achieve 1- 1/e of the final steady state value, which is 63% of the step increase to the final steady state value. To calculate the time constant of the response to 4% $CO_2$, the mean minute ventilation was calculated every ten seconds, and plotted against time.

An asymptotic curve was fitted, and the time taken to reach 63% of the magnitude of change in minute volume calculated.

The responses to the hypercarbic challenge were determined by:

1. The increase in minute volume in response to increased inspired $CO_2$
2. The increase in mean inspiratory flow in response to increased inspired $CO_2$
3. The time constant of the increase in minute volume in response to inspiring 4% $CO_2$

### 3.1.2 Exposure to smoking and substance misuse

Urine samples were obtained from all infants and mothers at the time of study for cotinine analysis and a drug screen. Urines were tested using assays on the CEDIA principle (Cloned Enzyme Donor ImmunoAssay), for cannabinoids, opiates, amphetamines, methadone, cocaine and benzodiazepines.

### 3.1.3 Data collection

Demographic and clinical data were gathered from the medical records. These were gestational age at birth, birth weight, mode of delivery, Apgar score, ethnicity, parity, maternally reported smoking and substance misuse during pregnancy. Individual birth weight centiles were calculated from gestational age, sex and birth weight based on a large UK dataset (Gardosi et al., 1995) using an online calculator (Gardosi J, 2010).

### 3.1.4 Analysis

Recruited infants were divided into those:

1. Infants of mothers who neither smoked nor misused substances during pregnancy (controls)
2. Infants of mothers who smoked, but did not misuse substances during pregnancy
3. Infants of mothers who misused substances during pregnancy

Differences between the three groups were assessed for statistical significance using the Kruskal-Wallis analysis of ranks test for continuous data or the Chi- square test for binary

data. Differences in the respiratory results between groups were assessed using regression analysis. Data were transformed as necessary using a square root transformation to meet regression assumptions. Adjustment was made for baseline differences in birth weight, gestational age, and postnatal age at study by fitting the variables as covariates. Results are presented as unadjusted and adjusted arithmetic means. Adjusted means are marginal estimates set to the mean value of the covariates.

Comparisons between prone and sleeping positions were made using the Paired samples t-test. Analyses were conducted using SPSS Version 22 (SPSS Inc., Chicago, IL, USA).

### 3.1.5 Sample size

Recruitment of twenty infants into each of the three groups allowed detection of a difference of one standard deviation in carbon dioxide sensitivity between the groups with 80% power and 5% significance. This magnitude of difference in the ventilatory response to added dead space between newborns of smoking and non-smoking mothers had been detected. (Bhat et al., 2005) The stimulus during added dead space is primarily hypercarbia.(Upton et al., 1992a).

## 3.2 Results

Ninety-one infants were recruited to the study. Twenty-eight infants did not complete the study protocol as the infant did not sleep (22 infants), or the family were discharged before the study could be performed (6 infants). There were no significant differences between those who were and were not studies with respect to birth weight, birth weight centile, gestational age or sex. (Table 3)

Sixty-three infants were studied in the newborn period. There were no significant differences between the three groups with regard to birth weight, birth weight centile, gestational age, sex and age of study. (Table 4)

### 3.2.1 Urine results

The urine drug screen was positive for all mothers in the substance-misusing group, (Table 5) and negative for all mothers and infants in the control and smoking group. Two infants in the substance-misusing group had a negative urine screen, but there was reported maternal use of amphetamines, benzodiazepines and ecstasy, which were not detected on urine screen. Four infants from the substance misusing group required treatment for withdrawal treatment with oral morphine, measurements were made prior to commencing opiate therapy.

All control mothers' and infants' urines were negative for cotinine and substances of misuse. All maternal urines in both the smoking group and substance misuse group were positive for cotinine (Table 6). Two infant urines in the smoking group and two in the substance misuse group were negative for cotinine.

| | Recruited and completed protocol | Recruited and did not complete protocol | p-value |
|---|---|---|---|
| **Number** | 63 | 28 | |
| **Birth weight (gms)** | 2890 (1950-4960) | 3190 (2340-3850) | 0.158 |
| **Gestational age (weeks)** | 39 (36-42) | 40 (36-41) | 0.102 |
| **Birth weight centile** | 13 (1-99) | 28 (1-99) | 0.18 |
| **Male (n)** | 31 | 18 | 0.25 |

Table 3: Demographics of infants recruited to the study comparing those that completed the protocol to those that did not. The data are demonstrated as median (range)

| | Controls | Smoking | Substance misuse | p-value |
|---|---|---|---|---|
| **Number recruited** | 36 | 35 | 20 | |
| **Number studied** | 22 | 23 | 18 | |
| **Birth weight (gms)** | 2950 (1980-4960) | 3060 (1950-3720) | 2880 (2010-4450) | 0.89 |
| **Gestational age (weeks)** | 39 (36-42) | 39 (36-42) | 39 (36-42) | 0.87 |
| **Birth weight centile** | 18 (1-99) | 12 (1-68) | 12 (1-93) | 0.56 |
| **Male (n)** | 7 | 13 | 11 | 0.28 |
| **Age at study (days)** | 3 (0-10) | 2 (1-7) | 3 (1-9) | 0.35 |

Table 4: Demographics of infants undergoing the hypercarbic challenge. The data are demonstrated as median (range)

| Substance | Number of mothers testing positive after delivery |
|---|---|
| **Opiates** | 14 |
| **Methadone** | 9 |
| **Morphine** | 11 |
| **Codeine** | 7 |
| **Cannabis** | 17 |
| **Cocaine metabolites** | 4 |

Table 5: Positive urine drug screens for mothers, some mothers were positive for several substances

| | Smoking group | Substance misuse group | p-value |
|---|---|---|---|
| **Infant urinary cotinine (ng/ml)** | 100 (0-3240) | 130 (0-1470) | 0.82 |
| **Maternal urinary cotinine (ng/ml)** | 1600 (20-46800) | 2420 (20-13000) | 0.83 |

Table 6: Infant and maternal urine cotinine results. The data are demonstrated as median (range)

Baseline cardiorespiratory characteristics differed significantly between the three groups in the supine position. Minute ventilation and respiratory rate were significantly higher in the substance misuse group compared to controls ($p=0.008$, $p<0.001$ respectively) and tidal volume significantly lower ($p=0.011$). Baseline respiratory rate was also significantly higher and tidal volume significantly lower in infants of substance misusing mothers compared to infants of smoking mothers ($p=0.002$, $p=0.017$ respectively) (Table 7). There were no significant differences in tidal volume, respiratory rate or minute volume between the controls and infants of smoking mothers. End tidal $CO_2$ while breathing air differed significantly between the groups ($p=0.011$). This difference was due to a significantly lower baseline end tidal $CO_2$ in the substance-misusing group compared to controls.

There were no significant differences in the baseline results between the three groups when studied in the prone position (Table 8).

There were no significant differences in the response to the hypercarbic challenge, between groups in the supine (Table 9) or prone (Table 10) position.

| | | Control | Smoking | Substance misuse | p-value |
|---|---|---|---|---|---|
| **Tidal Volume (ml/kg)** | Mean (SD; Range) Unadjusted | 7.9 (2.0; 5.5-15.2) | 7.6 (1.7; 4.8-10.8) | 6.4 (1.2; 4.5-8.7) | 0.023 |
| | Mean (95% CI), adjusted | 7.9 (7.3-8.5) | 7.6 (7.0-8.3) | 6.3 (5.7-7.0) | 0.002 |
| **Respiratory rate (breaths/min)** | Mean (SD; Range) Unadjusted | 46 (12; 21-75) | 52 (13; 34-77) | 69 (20; 39-110) | <0.001 |
| | Mean (95% CI), adjusted | 46 (40-53) | 52 (46-59) | 69 (62-76) | <0.001 |
| **Minute volume (ml/kg/min)** | Mean (SD; Range) Unadjusted | 336 (80; 232-501) | 373 (92; 220-600) | 434 (136; 220-782) | 0.017 |
| | Mean (95% CI), adjusted | 335 (291-379) | 376 (333-419) | 432 (385-478) | 0.015 |
| **Mean inspiratory flow (ml/kg/s)** | Mean (SD; Range) Unadjusted | 12.7(2.6; 8.5-16.5) | 14.0 (3.2; 9.6-20.9) | 14.7 (4.5; 8.8-28.3) | 0.182 |
| | Mean (95% CI), adjusted | 12.6 (11.2-14.0) | 14.1 (12.7-15.5) | 14.6 (13.1-16.1) | 0.140 |
| **End tidal $CO_2$ (%)** | Mean (SD; Range) Unadjusted | 4.7(0.5; 4.3-5.9) | 4.6 (0.5; 3.8-5.5) | 4.2 (0.6; 3.1-5.1) | 0.007 |
| | Mean (95% CI), adjusted | 4.7 (4.5-4.9) | 4.6 (4.3-4.8) | 4.2 (3.9-4.4) | 0.011 |
| **Heart rate (bpm)** | Mean (SD; Range) Unadjusted | 129 (10; 110-148) | 130 (16; 102-161) | 130 (17; 86-156) | 0.961 |
| | Mean (95% CI), adjusted | 128 (122-133) | 131 (125-137) | 131 (124-137) | 0.702 |

Table 7: Baseline cardiorespiratory characteristics while supine. Unadjusted results expressed as arithmetic mean (standard deviation; range). Results adjusted for birth weight, gestational age and postnatal age expressed as arithmetic mean and 95% confidence interval.

| | | Control | Smoking | Substance misuse | p-value |
|---|---|---|---|---|---|
| **Tidal Volume (ml/kg)** | Mean (SD; Range) Unadjusted | 7.7(1.3; 5.6-11.1) | 7.8 (2.0; 4.1-11.9) | 6.9 (1.5; 4.5-9.8) | 0.133 |
| | Mean (95% CI), adjusted | 7.8(7.1-8.5) | 7.8 (7.2-8.5) | 6.8 (6.1-7.5) | 0.056 |
| **Respiratory rate (breaths/min)** | Mean (SD; Range) Unadjusted | 45 (11; 26-69) | 47 (15; 29-72) | 54 (13; 30-81) | 0.101 |
| | Mean (95% CI), adjusted | 45 (39-51) | 48 (42-53) | 54 (48-60) | 0.112 |
| **Minute volume (ml/kg/min)** | Mean (SD; Range) Unadjusted | 342 (81; 211-486) | 360 (94; 228-537) | 348 (92; 219-593) | 0.820 |
| | Mean (95% CI), adjusted | 342 (303-381) | 361 (323-400) | 347 (308-386) | 0.769 |
| **Mean inspiratory flow (ml/kg/s)** | Mean (SD; Range) Unadjusted | 12.9 (3.3; 7.8-19.4) | 13.8 (3.0; 8.1-19.5) | 12.9 (2.8; 8.5- 18.2) | 0.605 |
| | Mean (95% CI), adjusted | 12.8 (11.5-14.2) | 13.9 (12.5-15.3) | 12.9 (11.5-14.2) | 0.481 |
| **End tidal $CO_2$ (%)** | Mean (SD; Range) Unadjusted | 4.8 (0.5; 4.1-6.3) | 4.6 (0.5; 3.9-5.5) | 4.5 (0.5; 3.3-5.6) | 0.264 |
| | Mean (95% CI), adjusted | 4.8 (4.6-5.0) | 4.7 (4.4-4.9) | 4.6 (4.3-4.8) | 0.382 |
| **Heart rate (bpm)** | Mean (SD; Range) Unadjusted | 132 (10; 115-159) | 130 (12; 108-155) | 130 (14; 100-155) | 0.766 |
| | Mean (95% CI), adjusted | 130 (126-135) | 132 (127-137) | 130 (125-136) | 0.909 |

Table 8: Baseline cardiorespiratory characteristics while prone. Unadjusted results expressed as arithmetic mean (standard deviation; range). Results adjusted for birth weight, gestational age and postnatal age expressed as arithmetic mean and 95% confidence interval.

| | | Controls | Smoking | Substance misuse | p-value Between groups |
|---|---|---|---|---|---|
| **$CO_2$ sensitivity (minute volume) (ml/kg/min/% $CO_2$)** | Mean (SD; Range) Unadjusted | 37 (30; -14-88) | 34 (34; -13-132) | 33 (22; -3-80) | 0.904 |
| | Mean (95% CI), adjusted | 38 (25-51) | 34 (21-47) | 33 (19-46) | 0.840 |
| **$CO_2$ sensitivity (mean inspiratory flow) (ml/kg/s/% $CO_2$)** | Mean (SD; Range) Unadjusted | 1.5 (1.2; -0.4-3.8) | 1.3 (1.0; -0.4-3.9) | 1.1 (0.8; -1.0- 2.3) | 0.456 |
| | Mean (95% CI), adjusted | 1.5 (1.0-1.9) | 1.3 (0.9-1.8) | 1.0 (0.6-1.5) | 0.417 |
| **Time constant (s)** | Mean (SD; Range) Unadjusted | 37 (13;2-218) | 44 (12.9; 3-234) | 41 (12.3; 1-126) | 0.897 |
| | Mean (95% CI), adjusted | 37 (20-60) | 44 (25-69) | 41 (22-68) | 0.904 |

Table 9: Results of hypercarbic challenge in the supine position according to group. Unadjusted results expressed as arithmetic mean (standard deviation; range). Results adjusted for birth weight, gestational age and postnatal age expressed as arithmetic mean and 95% confidence interval.

| | | Controls | Smoking | Substance misuse | p-value Between groups |
|---|---|---|---|---|---|
| **$CO_2$ sensitivity (minute volume) (ml/kg/min/% $CO_2$)** | Mean (SD; Range) Unadjusted | 35 (22; 4-81) | 38 (29; -16-80) | 42 (21; 3-87) | 0.658 |
| | Mean (95% CI), adjusted | 35 (24-47) | 38 (26-49) | 42 (31-53) | 0.713 |
| **$CO_2$ sensitivity (mean inspiratory flow) (ml/kg/s/% $CO_2$)** | Mean (SD; Range) Unadjusted | 1.3 (0.8; 0.2-3.1) | 1.3 (1.0; -1.0-3.1) | 1.4 (0.7; 0.2-3.1) | 0.906 |
| | Mean (95% CI), adjusted | 1.3 (0.9-1.7) | 1.3 (0.9-1.7) | 1.4 (1.0-1.8) | 0.952 |
| **Time constant (s)** | Mean (SD; Range) Unadjusted | 46 (14;10-240) | 54 (11; 9-100) | 67 (12; 7-280) | 0.519 |
| | Mean (95% CI), adjusted | 49 (27-77) | 52 (31-79) | 66 (42-96) | 0.614 |

Table 10: Results of hypercarbic challenge in the prone position according to group. Unadjusted results expressed as arithmetic mean (standard deviation; range). Results adjusted for birth weight, gestational age and postnatal age expressed as arithmetic mean and 95% confidence interval.

Sleeping position had no significant effect on either the baseline results or the response to hypercarbic challenge in control infants (Table 11).

In infants of smoking mothers, respiratory rate was significantly lower in the prone compared to the supine position (p=0.042) (Table 12). There was no significant effect of sleeping position on the ventilatory response to hypercarbia.

Infants of substance misusing mothers had a significantly lower respiratory rate in the prone compared to supine position (p=0.001) and the minute ventilation was significantly lower in the prone compared to supine position (p=0.005). The end-tidal $CO_2$ was significantly higher in the prone position (p=0.006) (Table 13).

| | Supine | Prone | p-value |
|---|---|---|---|
| **Tidal Volume (ml/kg)** | 7.9 (2.0; 5.5-15.2) | 7.7(1.3; 5.6-11.1) | 0.615 |
| **Respiratory rate (breaths/min)** | 46 (12; 21-75) | 45 (11; 26-69) | 0.688 |
| **Baseline minute volume (ml/kg/min)** | 336 (80; 232-501) | 342 (81; 211-486) | 0.906 |
| **Baseline Mean inspiratory flow (ml/kg/s)** | 12.7(2.6; 8.5-16.5) | 12.9 (3.3; 7.8-19.4) | 0.973 |
| **End tidal $CO_2$ (%)** | 4.7(0.5; 4.3-5.9) | 4.8 (0.5; 4.1-6.3) | 0.436 |
| **Heart rate (bpm)** | 129 (10; 110-148) | 132 (10; 115-159) | 0.180 |
| **$CO_2$ sensitivity (minute volume) (ml/kg/min/% $CO_2$)** | 37 (30; -14-88) | 35 (22; 4-81) | 0.771 |
| **$CO_2$ sensitivity (mean inspiratory flow) (ml/kg/s/% $CO_2$)** | 1.5 (1.2; -0.4-3.8) | 1.3 (0.8; 0.2-3.1) | 0.465 |
| **Time constant (s)** | 37 (13;2-218) | 46 (14;10-240) | 0.140 |

Table 11: Baseline measurements and hypercarbic challenge results in prone and supine position, control group. Data presented as arithmetic mean (standard deviation; range)

| | Supine | Prone | p-value |
|---|---|---|---|
| **Tidal Volume (ml/kg)** | 7.6 (1.7; 4.8-10.8) | 7.8 (2.0; 4.1-11.9) | 0.209 |
| **Respiratory rate (breaths/min)** | 52 (13; 34-77) | 47 (15; 29-72) | 0.042 |
| **Baseline minute volume (ml/kg/min)** | 373 (92; 220-600) | 360 (94; 228-537) | 0.388 |
| **Baseline Mean inspiratory flow (ml/kg/s)** | 14.0 (3.2; 9.6-20.9) | 13.8 (3.0; 8.1-19.5) | 0.764 |
| **End tidal $CO_2$ (%)** | 4.6 (0.5; 3.8-5.5) | 4.6 (0.5; 3.9-5.5) | 0.268 |
| **Heart rate (bpm)** | 130 (16; 102-161) | 130 (12; 108-155) | 0.538 |
| **$CO_2$ sensitivity (minute volume) (ml/kg/min/% $CO_2$)** | 34 (34; -13-132) | 38 (29; -16-80) | 0.825 |
| **$CO_2$ sensitivity (mean inspiratory flow) (ml/kg/s/% $CO_2$)** | 1.3 (1.0; -0.4-3.9) | 1.3 (1.0; -1.0-3.1) | 0.776 |
| **Time constant (s)** | 44 (12.9; 3-234) | 54 (11; 9-100) | 0.676 |

Table 12: Baseline measurements and hypercarbic challenge results in prone and supine position, smoking group. Data presented as arithmetic mean (standard deviation; range)

| | Supine | Prone | p-value |
|---|---|---|---|
| **Tidal Volume (ml/kg)** | 6.4 (1.2; 4.5-8.7) | 6.9 (1.5; 4.5-9.8) | 0.063 |
| **Respiratory rate (breaths/min)** | 69 (20; 39-110) | 54 (13; 30-81) | 0.001 |
| **Baseline minute volume (ml/kg/min)** | 434 (136; 220-782) | 348 (92; 219-593) | 0.005 |
| **Baseline Mean inspiratory flow (ml/kg/s)** | 14.7 (4.5; 8.8-28.3) | 12.9 (2.8; 8.5-18.2) | 0.08 |
| **End tidal $CO_2$ (%)** | 4.2 (0.6; 3.1-5.1) | 4.5 (0.5; 3.3-5.6) | 0.006 |
| **Heart rate (bpm)** | 130 (17; 86-156) | 130 (14; 100-155) | 0.806 |
| **$CO_2$ sensitivity (minute volume) (ml/kg/min/% $CO_2$)** | 33 (22; -3-80) | 42 (21; 3-87) | 0.191 |
| **$CO_2$ sensitivity (mean inspiratory flow) (ml/kg/s/% $CO_2$)** | 1.1 (0.8; -1.0- 2.3) | 1.4 (0.7; 0.2-3.1) | 0.196 |
| **Time constant (s)** | 41 (12.3; 1-126) | 67 (12; 7-280) | 0.183 |

Table 13: Baseline measurements and hypercarbic challenge results in prone and supine position, substance misuse group. Data presented as arithmetic mean (standard deviation; range)

## 3.3 Discussion

In this study I have demonstrated significant differences in baseline ventilation both between infants exposed to smoking and substance misuse in pregnancy and controls, and between the prone and supine sleeping position. This was a significantly higher respiratory rate and lower tidal volumes in the infants exposed to substance misuse in pregnancy. A possible explanation for these findings is that the infants were withdrawing from substance exposure. An increased respiratory rate has been reported in withdrawing infants (Glass et al., 1972, Ali et al., 2014) and explanations include increased metabolic demands in withdrawing infants, or increased agitation and activity. I found a compensatory reduction in tidal volume that partially offset the effect on minute volume. Nonetheless the end tidal $CO_2$ was significantly lower in infants of substance misusing mothers, suggesting that the increased minute volume and respiratory rate is not driven solely by increased metabolic demands and increased $CO_2$ production. In neonatal rat studies, the primary effect of opiate exposure is a reduction in respiratory frequency with minimal effect on tidal volume,(Greer et al., 1995) similarly in adult humans undergoing anaesthetic the effect is primarily on respiratory rate until opiate doses are very high.(Pattinson, 2008) Withdrawal of opiates is associated with an increased respiratory rate in adults.(Glass et al., 1972)

The finding of differences in baseline ventilation between groups is consistent with other studies evaluating respiratory control in infants exposed to substances in-utero. Glass et al. measured the respiratory rate and blood gases of 22 infants born to heroin addicted mothers and compared them to unexposed controls matched by gestation and birth weight. The heroin exposed infants had significantly higher respiratory rates, with a higher pH on blood gas compared to controls.(Glass et al., 1972) No measurements of tidal volume were included. Ali et al. reported significantly higher minute volumes and respiratory rates in infants of substance misusing infants and infants of smoking mothers compared to controls. This contrasts with the present results, in that there was no significant difference in baseline ventilation between controls and those exposed to smoking in our study.(Ali et al., 2014) In contrast, Wingkun et al. found no difference in baseline respiratory characteristics between 12 term infants born to substance misusing mothers, and 12 controls.(Wingkun et al., 1995) The infants, however,

were mostly studied within the first twenty-four hours after birth and, therefore, may not have exhibited evidence of withdrawal at the time of measurement.(32)

Prone sleeping was associated with a significantly lower respiratory rate, minute volume, and consequently higher end-tidal $CO_2$ when compared to sleeping supine in infants exposed to substance misuse in-utero. In infants of smoking mothers, the respiratory rate was significantly lower in the prone compared to supine position, however, this did not result in a significant difference in minute volume or end-tidal $CO_2$ between the prone and supine sleeping position. No significant differences were seen in the control group. Smith et al. found no significant difference in minute volume, tidal volume or respiratory rate between the prone and supine sleeping position in eighteen convalescent preterm infants that had not been exposed to substance misuse in-utero.(Smith et al., 2010) Prone sleeping has been shown to result in a greater functional residual capacity with improved oxygenation compared to the supine sleeping in convalescent preterm infants.(Kassim et al., 2007) In eighteen prematurely born infants (median gestational age 30 weeks) a stronger Hering-Breuer inflation reflex was demonstrated in the prone compared to supine position, which correlated strongly with the increased FRC in this position.(Landolfo et al., 2008) This vagally mediated reflex prevents excessive inflation and stretch of the lung. In the prone position it may exert an inhibitory effect on respiratory drive.

The results presently describe the differences in baseline ventilation between the prone and supine position in infants of substance misusing mothers are suggestive of damping of respiratory drive in the prone position, as opposed to increased efficacy of gas exchange in that position, as I have detected a significantly higher end-tidal $CO_2$ in the prone position.

This study demonstrated no difference in the response to hypercarbic challenge between controls, infants of mothers who smoked in pregnancy and infants of mothers who misused substances. Nor did sleeping position influence the response to hypercarbia in any of the groups. The results of this study differ from other studies. Ali et al. demonstrated significantly damped ventilatory responses to hypercarbia in infants of smoking and substance misusing mothers compared to controls.(Ali et al., 2014) In the study by Ali et al. birth weight and gestation differed significantly between the infants of substance misusing, smoking and control

mothers whereas there were no significant differences seen in our study. While the control and infants of smoking mothers were of similar gestation and birth weight in the two studies, the infants of substance misusing mothers were born at a median gestation two weeks less in the study by Ali et al. compared to this study. Accounting for this by calculating the birth weight centiles, there was a trend towards infants in the substance misuse group having a lower birth weight centile in the study by Kamal et al. (p=0.09). There was no difference between birth weight centiles in our study suggesting that any impact on fetal growth by substance misuse may be less in our study.

A comparison of infant urinary cotinine levels demonstrated significantly higher cotinine levels in Ali's study compared to those in this study (median 145ng/ml range (11-8760) vs 130ng/ml (0-3240) p=0.036). Greater exposure to smoking in the earlier study may account for the differing findings when infants were studied in the supine position. Post-mortem studies of the brainstems of infants who have died of SIDS have consistently demonstrated abnormalities of serotonergic receptor binding compared to infants dying of other causes.(Duncan et al., 2010) Serotonergic transmission plays a crucial role in the detection and response to hypercarbia,(Bianchi and Gestreau, 2009) and induced mutations in rats result in impaired ventilatory and arousal responses to hypercarbia.(Buchanan and Richerson, 2010) Antenatal exposure to nicotine results in abnormalities in brainstem serotonergic transporter expression.(Muneoka et al., 2001) It is plausible that a greater exposure to nicotine in-utero may have a more deleterious effect on ventilatory response to hypercarbia.

In addition a greater proportion of infants of substance misusing mothers in the study by Ali et al. went on to require treatment for neonatal abstinence (9/21 compared to 4 /17 infants; p=0.3). This suggests a greater degree of exposure to opiates in the infants enrolled in the previous study.

Wingkun et al. demonstrated a dampened response to hypercarbia in infants of substance-misusing mothers compared to controls in the neonatal period.(Wingkun et al., 1995) Olsen measured nine infants of methadone users in the neonatal period, and found a significantly damped ventilatory response to hypercarbia compared to non-exposed controls, which persisted for the first two weeks of life.(Olsen and Lees, 1980) In guinea pigs in-utero

exposure to morphine or methadone was shown to increase the ventilatory response to hypercarbia on day three after birth, which persisted to day seven in those exposed to morphine, before returning to the same level of response as controls by day 14 after birth.(Nettleton et al., 2008) In this study I found no difference in the response to hypercarbic challenge between the prone and supine position in any of the three groups. Several studies have found evidence of impaired respiratory drive in the prone compared to supine position. Smith et al. studied the effect of prone sleeping position on the ventilatory responses to hypercarbia by assessing the pressures generated during an occlusion during the first 100 milliseconds (P0.1) and the maximum pressure generated against an occlusion while crying (Pimax). A weaker response was generated in the prone position suggesting reduced respiratory drive.(Smith et al., 2010) Saiki et al. introduced added dead space as a respiratory challenge and found the time constant of the response was prolonged in the prone compared to the supine position, again suggesting a damped response.(Saiki et al., 2010) Both studies were performed on convalescing prematurely born infants. In a further study of eighteen convalescent, preterm infants studied post-term exposed to a hypercarbic challenge in both the prone and supine position, Saiki et al. demonstrated a higher functional residual capacity and lower p0.1/Pimax in the prone compared to supine position, but found no difference in the ventilatory response to hypercarbia.(Saiki et al., 2014) Galland et al. used a mixed asphyxia challenge in infants from the neonatal period into the high risk period for SIDS (3 months) and found a damped response to asphyxia gas in the prone position, but only at 3 months of age, and this was also associated with a heightened arousal response.(Galland et al., 2000) Conversely, Martin et al. found the supine position was associated with a smaller increase in minute volume in response to a hypercarbic challenge when compared to the prone position in nineteen healthy preterm infants approaching discharge. (Martin et al., 1995) The discrepancy between these results may reflect the differing patient groups, as most studies were performed on convalescing preterm infants. The maturation of the Hering-Breuer reflex has been demonstrated to be delayed in infants born preterm,(Stocks et al., 1996) such that prematurely born infants studied at term differ significantly from term newborns. Differing results between these populations may therefore be expected. It is of interest that when prematurely born and term infants were studied at the same post-conceptual age, the results did not differ significantly, in keeping with the finding that the high risk age for SIDS in prematurely born

infants is at a consistent post-conceptual age, but a later postnatal age in more preterm infants. (Halloran and Alexander, 2006)

This study has strengths and some limitations. The smoking and substance use of mothers was confirmed by urine analysis rather than maternal report alone. The protocol required a minimum of an hour of measurements, which was prolonged when the infant aroused. On these occasions the infants were allowed to settle back into quiet sleep before measurements were repeated. Several infants did not tolerate the measurements and these were excluded from analysis which may introduce a degree of bias, with measurement favouring those with poorer arousal responses or less irritable.

End-tidal $CO_2$ was measured as described in Chapter 2 using a side-stream sampling technique. This allowed both the dead space of the equipment, and the weight and discomfort to the baby to be minimised. This technique draws respiratory gas from the nasal mask at a constant rate, for analysis remotely from the infant. At lower minute volumes this could potentially draw non-expired air into the sampling line underestimating end-tidal $CO_2$. However, visual evaluation of the expiratory waveform consistently demonstrated stable end-expiratory plateau suggesting exclusively expired gas was being sampled.

Measurement of $CO_2$ sensitivity produced results with a large standard deviation. Although every effort was made to ensure that measurements were made only during quiet sleep, it is possible that subclinical arousals may have contributed to variation in minute volume at different levels of inspired carbon dioxide. Irrespective of cause, the magnitude of the standard error of the results may account for the failure to detect the difference in carbon dioxide sensitivity demonstrated by Ali et al. (Ali et al., 2014)

In conclusion, this study has demonstrated significant differences in baseline ventilation in the prone compared to supine position in infants of smoking mothers and substance misusing mothers, suggestive of reduced respiratory drive when sleeping prone. This would support the argument that prone sleeping is a risk factor for SIDS rather than suffocation.(Lewak, 2012) The effect of prone sleeping was significant only in the smoking and substance misusing groups, suggesting prone sleeping may be of particular risk when associated with these risk factors.

# Chapter 4 : The effects of sleeping position and maternal smoking and substance misuse on the ventilatory response to hypoxia in the newborn period

Maternal smoking (Zhang and Wang, 2013) and substance misuse during pregnancy (Ward et al., 1990) and infant prone sleeping (Oyen et al., 1997) all increase the risk of SIDS.

Maternal smoking during pregnancy has been reported to increase SIDS risk, and a meta-analysis of 35 case-control studies estimated an approximately two-fold increase in risk of SIDS in infants of mothers who smoked in pregnancy compared to controls (OR = 2.25, 95% CI = 2.03–2.50).(Zhang and Wang, 2013) Furthermore the risk was dose dependent, with heavier smoking mothers having infants at greater risk. The increased risk of SIDS in infants of substance abusing mothers (ISAMs) compared to controls has been reported from four to eight-fold.(Kandall et al., 1993, Ward et al., 1990) In a case control study the odds ratio of SIDS having been placed prone to sleep was 13.9 compared to being placed supine.(Oyen et al., 1997) The effect of a combination of risk factors may be more than additive. Oyen et al. in the Nordic Epidemiological SIDS study found an odds ratio (OR) of 4.9 in infants of mothers who smoked in pregnancy compared to controls sleeping supine, however the OR increased to 55 when the combination of maternal smoking in pregnancy and prone sleeping were present.(Oyen et al., 1997) The interaction of other risk factors including low birth weight and prematurity with prone sleeping also resulted in a multiplicative increase in risk of SIDS. The combined risk of prone sleeping and maternal substance misuse is unreported.

In this study, I have tested the hypothesis that the ventilatory response to hypoxia would be more damped in infants of smoking and/or substance misusing mothers compared to that of controls, particularly in the prone compared to supine position.

## 4.1 Methods

Infants were eligible for inclusion in the study if they were born at 36 weeks of gestation or greater at King's College Hospital NHS Foundation Trust. They were studied prior to discharge from the maternity unit. Exclusion criteria were major congenital abnormalities, respiratory disease or sepsis. Approval for the study was obtained from the London – Bromley Research Ethics Committee. Parents gave informed written consent for their infant to participate.

### 4.1.1 Protocol

Infants were studied prior to maternity unit/neonatal unit discharge at between 36 and 42 weeks corrected gestational age. After a feed, the infant was placed in either the prone or supine sleeping position, the other position being studied afterwards on the same day. The order in which the positions were studied was randomised between infants. Measurements were made while the infant was in quiet sleep. Sleep state was determined by observation of the behavioural state.(Prechtl, 1974) If arousal occurred the measurement was abandoned and recommenced once the infant returned to quiet sleep. The hypoxic challenge was delivered via a nasal mask and open circuit system as described in Chapter 2.

Data were averaged over 10 second periods and these averaged data points were used to quantify the initial increase in ventilation in response to hypoxia, and the timing and magnitude of the subsequent decline in ventilation in response to sustained hypoxia.

1. The responses to the hypoxic challenge were determined by
2. The time from the start of hypoxic challenge to the peak ventilation
3. The magnitude of increase in minute ventilation from baseline to the peak ventilation
4. The magnitude of decline in minute volume from the peak to the lowest minute volume
5. The rate of decline in minute volume, calculated as the peak minute volume-lowest minute volume divided by the time from peak to the lowest minute volume.

6. The change in the oxygen saturation level from baseline to the lowest oxygen saturation level. The challenge was abandoned if oxygen saturations dropped below 85%.

### 4.1.2 Exposure to smoking and substance misuse

Urine samples were obtained from all infants and mothers at the time of study for cotinine analysis and assessment of maternal substance misuse.

### 4.1.3 Data collection

Demographic and clinical data were gathered from the medical records. These were gestational age at birth, birth weight, mode of delivery, Apgar score, ethnicity, parity, maternally reported smoking and substance misuse during pregnancy. Individual birth weight centiles were calculated from gestational age, sex and birth weight based on a large UK dataset(Gardosi et al., 1995) using an online calculator(Gardosi J, 2010).

### 4.1.4 Analysis

Recruited infants were divided into three groups:

1. Infants of mothers who neither smoked nor misused substances during pregnancy (controls)
2. Infants of mothers who smoked, but did not misuse substances during pregnancy
3. Infants of mothers who misused substances during pregnancy

Differences between the three groups were assessed for statistical significance using the Kruskal-Wallis analysis of ranks test or the Chi- square test as appropriate. Differences in the responses to the hypoxic challenge between groups were assessed using regression analysis. Data were transformed as necessary using a square root or logarithmic transformation to meet regression assumptions. Adjustment was made for baseline differences in birth weight, gestational age and postnatal age at study by fitting the variables as covariates. Results are

presented as unadjusted and adjusted arithmetic means, or geometric means. Adjusted means are marginal estimates set to the mean value of the covariates. Comparisons between prone and sleeping positions were made using the Paired samples t-test. Analyses were conducted using SPSS Version 22 (SPSS Inc., Chicago, IL, USA).

#### 4.1.5 Sample size

Recruitment of twenty infants into each of the three groups allowed detection of a difference of one standard deviation in each outcome between the groups with 80% power at the 5% level. That magnitude of difference had been detected in the ventilatory response to added dead space between newborns of smoking and non-smoking mothers.(Bhat et al., 2005)

## 4.2 Results

Ninety-one infants were recruited to the study. Twenty-eight infants did not complete the study protocol as the infant did not sleep (22 infants) or the family were discharged before the study could be performed (6 infants). There were no significant differences between those who were and were not studies with respect to birth weight, birth weight centile, gestational age or sex (Table 14).

Sixty-three infants were studied in the newborn period. There were no significant differences between the three groups with regard to birth weight, birth weight centile, gestational age, sex and age of study (Table 15).

### 4.2.1 Urine results

The urine drug screen was positive for all mothers in the substance-misusing group,(Table 16) and negative for all mothers and infants in the control and smoking group. Two infants in the substance-misusing group had a negative urine screen, but there was reported maternal use of amphetamines, benzodiazepines and ecstasy that were not detected on urine screen. Four infants from the substance misusing group required treatment for withdrawal treatment with oral morphine, measurements were made prior to commencing opiate therapy.

All control mothers' and infants' urines were negative for cotinine and substances of misuse. All maternal urines in both the smoking group and substance misuse group were positive for cotinine (Table 17). Two infant urines in the smoking group and two in the substance misuse group were negative for cotinine.

| | Recruited and completed protocol | Recruited and did not complete protocol | p-value |
|---|---|---|---|
| **Number** | 63 | 28 | |
| **Birth weight (gms)** | 2890 (1950-4960) | 3190 (2340-3850) | 0.158 |
| **Gestational age (weeks)** | 39 (36-42) | 40 (36-41) | 0.102 |
| **Birth weight centile** | 13 (1-99) | 28 (1-99) | 0.18 |
| **Male (n)** | 31 | 18 | 0.25 |

Table 14: Demographics of infants recruited to the study comparing those that completed the protocol to those that did not. The data are demonstrated as median (range)

| | Controls | Smoking | Substance misuse | p-value |
|---|---|---|---|---|
| **Number recruited** | 36 | 35 | 20 | |
| **Number studied** | 22 | 23 | 18 | |
| **Birth weight (gms)** | 2950 (1980-4960) | 3060 (1950-3720) | 2880 (2010-4450) | 0.89 |
| **Gestational age (weeks)** | 39 (36-42) | 39 (36-42) | 39 (36-42) | 0.87 |
| **Birth weight centile** | 18 (1-99) | 12 (1-68) | 12 (1-93) | 0.56 |
| **Male (n)** | 7 | 13 | 11 | 0.28 |
| **Age at study (days)** | 3 (0-10) | 2 (1-7) | 3 (1-9) | 0.35 |

Table 15: Demographics of infants undergoing the hypoxic challenge. The data are demonstrated as median (range)

| Substance | Number of mothers testing positive after delivery |
|---|---|
| **Opiates** | 14 |
| **Methadone** | 9 |
| **Morphine** | 11 |
| **Codeine** | 7 |
| **Cannabis** | 17 |
| **Cocaine metabolites** | 4 |

Table 16: Positive urine drug screens for mothers, some mothers were positive for several substances

| | Smoking group | Substance misuse group | p-value |
|---|---|---|---|
| **Infant urinary cotinine (ng/ml)** | 100 (0-3240) | 130 (0-1470) | 0.82 |
| **Maternal urinary cotinine (ng/ml)** | 1600 (20-46800) | 2420 (20-13000) | 0.83 |

Table 17: Infant and maternal urine cotinine results. The data are demonstrated as median (range)

In the supine position while breathing air tidal volume, respiratory rate and minute volume differed significantly between the three groups. (Table 7) Minute ventilation and respiratory rate were significantly higher in the substance misuse group compared to controls ($p=0.008$, $p<0.001$ respectively) and tidal volume significantly lower ($p=0.011$). Baseline respiratory rate was also significantly higher and tidal volume significantly lower in infants of substance misusing mothers compared to infants of smoking mothers ($p=0.002$, $p=0.017$ respectively).

In the supine position there were no significant differences between the groups in any of the measures of the hypoxic ventilatory response. (Table 18)

In the prone position, the hypoxic rate of decline differed significantly across the three groups ($p=0.002$). There was a significantly greater rate of decline in the infants of substance misusing mothers compared to controls ($p=0.002$) and between infants of substance misusing mothers and infants of smoking mothers ($p=0.016$). (Table 19) These differences remained significant after adjusting for gestation at birth, postnatal age and birth weight.

| | | Controls | Smoking | Substance misuse | p-value Between groups |
|---|---|---|---|---|---|
| **Hypoxic increase in minute ventilation (ml/kg/min)** | Mean (SD†; Range) Unadjusted | 69 (65; 0-197) | 82 (84; 0-290) | 71 (67; 0-186) | 0.95 |
| | Mean (95% CI), adjusted‡ | 43 (18-79) | 59 (30-98) | 47 (20-86) | 0.77 |
| **Time to peak minute ventilation during hypoxic challenge (s)** | Mean (SD†; Range) Unadjusted | 109 (55; 26-214) | 97 (62; 10-218) | 153 (108; 16-380) | 0.08 |
| | Mean (95% CI), adjusted‡ | 108 (73-143) | 100 (66-134) | 151 (114-188) | 0.10 |
| **Hypoxic rate of decline in ventilation (ml/kg/min/s) *** | Mean (SD†; Range) Unadjusted | 3.9 (6.9; 0-23) | 3.6 (6.7; 0-27) | 4.2 (10.9; 0-36) | 0.93 |
| | Mean (95% CI), adjusted‡ | 3.5 (1.9-6.0) | 3.9 (2.3-6.4) | 4.1 (2.3-7.0) | 0.92 |
| **Magnitude of decline (ml/kg/min)** | Mean (SD†; Range) Unadjusted | 130 (84; -40-270) | 148 (156; -50-540) | 155 (117; 10-380) | 0.84 |
| | Mean (95% CI), adjusted‡ | 123 (65-182) | 153 (100-206) | 155 (97-214) | 0.69 |
| **Minimum saturations (%)** | Mean (SD†; Range) Unadjusted | 90 (5; 84-98) | 89 (5; 81-98) | 89(5; 81-97) | 0.63 |
| | Mean (95% CI), adjusted‡ | 90 (88-92) | 89 (87-91) | 89 (87-91) | 0.56 |

Table 18: Results of hypoxic challenge in the supine position according to group.

*Denotes that all means are geometric means due to skewness of the distribution.

†SD on natural scale.

‡Denotes marginal means adjusted for birth weight, gestational age, and postnatal age (set to mean values).

| | | Controls | Smoking | Substance misuse | p-value Between groups |
|---|---|---|---|---|---|
| **Hypoxic increase in minute ventilation (ml/kg/min)** | Mean (SD†; Range) Unadjusted | 94 (86; 0-260) | 62 (63; 0-240) | 68 (58; 0-170) | 0.32 |
| | Mean (95% CI), adjusted‡ | 75 (43-114) | 42 (20-72) | 49 (25-82) | 0.31 |
| **Time to peak minute ventilation during hypoxic challenge (s)** | Mean (SD†; Range) Unadjusted | 137 (91; 4-330) | 103 (85; 6-348) | 137 (94; 4-270) | 0.41 |
| | Mean (95% CI), adjusted‡ | 133 (90-177) | 106 (64-149) | 136 (93-180) | 0.55 |
| **Hypoxic rate of decline in ventilation (ml/kg/min/s)*** | Mean (SD†; Range) Unadjusted | 1.5 (2.0; 0-7) | 2.1 (3.2; 0-10) | 5.6 (14.5; 1-57) | 0.002 |
| | Mean (95% CI), adjusted‡ | 1.5 (0.7-3.6) | 2.1 (1.2-4.5) | 6.4 (4.4-9.5) | 0.002 |
| **Magnitude of decline (ml/kg/min)** | Mean (SD†; Range) Unadjusted | 121 (115; -30-360) | 184 (86; 40-320) | 189 (111; 40-430) | 0.13 |
| | Mean (95% CI), adjusted‡ | 121 (70-172) | 185 (134-236) | 186 (133-240) | 0.14 |
| **Minimum saturations (%)** | Mean (SD†; Range) Unadjusted | 89 (5; 81-98) | 89 (5; 78-97) | 91 (5; 82-99) | 0.52 |
| | Mean (95% CI), adjusted‡ | 89 (87-91) | 89 (86-91) | 91 (88-94) | 0.51 |

Table 19: Results of hypoxic challenge in the prone position according to group.

*Denotes that all means are geometric means due to skewness of the distribution.

†SD on natural scale.

‡Denotes marginal means adjusted for birth weight, gestational age, and postnatal age (set to mean values).

In the controls, the hypoxic rate of decline was greater in the supine compared to the prone position ($p=0.02$). (Table 20)

In neither the smoking (Table 21) nor the substance misuse (Table 22) group were there significant differences in the results between the supine and prone positions.

The differences between the responses in the prone and supine position did not differ significantly between the groups. (Table 23)

| | Supine | Prone | p-value |
|---|---|---|---|
| **Hypoxic increase in minute ventilation (ml/kg/min)** | 69 (65; 0-197) | 94 (86; 0-260) | 0.18 |
| **Time to peak minute ventilation during hypoxic challenge (s)** | 109 (55; 26-214) | 137 (91; 4-330) | 0.29 |
| **Hypoxic rate of decline in ventilation (ml/kg/min/s)*** | 3.9 (6.9; 0-23) | 1.5 (2.0; 0-7) | 0.02 |
| **Magnitude of decline (ml/kg/min)** | 130 (84; -40-270) | 121 (115; -30-360) | 0.77 |
| **Minimum saturations (%)** | 90 (5; 84-98) | 89 (5; 81-98) | 0.50 |

Table 20: Baseline measurements and hypoxic challenge results in prone and supine position, control group. Data presented as arithmetic mean (standard deviation on natural scale; range) except * denotes that means are geometric means due to skewness of the distribution.

| | Supine | Prone | p-value |
|---|---|---|---|
| **Hypoxic increase in minute ventilation (ml/kg/min)** | 82 (84; 0-290) | 62 (63; 0-240) | 0.49 |
| **Time to peak minute ventilation during hypoxic challenge (s)** | 97 (62; 10-218) | 103 (85; 6-348) | 0.74 |
| **Hypoxic rate of decline in ventilation (ml/kg/min/s)*** | 3.6 (6.7; 0-27) | 2.1 (3.2; 0-10) | 0.40 |
| **Magnitude of decline (ml/kg/min)** | 148 (156; -50-540) | 184 (86; 40-320) | 0.13 |
| **Minimum saturations (%)** | 89 (5; 81-98) | 89 (5; 78-97) | 0.94 |

Table 21: Baseline measurements and hypoxic challenge results in prone and supine position, smoking group. Data presented as arithmetic mean (standard deviation on natural scale; range) except * denotes that means are geometric means due to skewness of the distribution.

| | Supine | Prone | p-value |
|---|---|---|---|
| **Hypoxic increase in minute ventilation (ml/kg/min)** | 71 (67; 0-186) | 68 (58; 0-170) | 0.86 |
| **Time to peak minute ventilation during hypoxic challenge (s)** | 153 (108; 16-380) | 137 (94; 4-270) | 0.67 |
| **Hypoxic rate of decline in ventilation (ml/kg/min/s)*** | 4.2 (10.9; 0-36) | 5.6 (14.5; 1-57) | 0.39 |
| **Magnitude of decline (ml/kg/min)** | 155 (117; 10-380) | 189 (111; 40-430) | 0.67 |
| **Minimum saturations (%)** | 89(5; 81-97) | 91 (5; 82-99) | 0.42 |

Table 22: Baseline measurements and hypoxic challenge results in prone and supine position, substance misuse group. Data presented as arithmetic mean (standard deviation on natural scale; range) except * denotes that means are geometric means due to skewness of the distribution.

| | | Controls | Smoking | Substance misuse | p-value Between groups |
|---|---|---|---|---|---|
| **Hypoxic increase in minute ventilation (ml/kg/min)** | Mean (SD†; Range) Unadjusted | -33 (110; -300-111) | 31 (110; -170-290) | 21 (65; -88-125) | 0.20 |
| | Mean (95% CI), adjusted‡ | -36 (-80-8) | 25 (-17-68) | 5 (-39-48) | 0.14 |
| **Time to peak minute ventilation during hypoxic challenge (s)** | Mean (SD†; Range) Unadjusted | -22 (97;-72-118) | -30 (84; -132-196) | 47 (169; -202-266) | 0.60 |
| | Mean (95% CI), adjusted‡ | -21 (-79-37) | -9 (-65-48) | 15 (-42-73) | 0.66 |
| **Hypoxic rate of decline in ventilation (ml/kg/min/s)*** | Mean (SD†; Range) Unadjusted | 4.6 (6.5; -3.9-18.3) | 1.5 (7.5; -7.7-25.4) | -2.4 (8.7; -21-5.8) | 0.12 |
| | Mean (95% CI), adjusted‡ | 4.4 (-0.1-8.9) | 1.6 (-2.8-6.0) | -1.8 (-6.5-2.8) | 0.17 |
| **Magnitude of decline (ml/kg/min)** | Mean (SD†; Range) Unadjusted | 10.4 (137; -280-250) | -55(140; -300-186) | -39 (155; -250-181) | 0.46 |
| | Mean (95% CI), adjusted‡ | 10.3 (-66-87) | -52 (-126-22) | -20 (-98-59) | 0.51 |
| **Minimum saturations (%)** | Mean (SD†; Range) Unadjusted | 2.5 (6.3; -11-16) | 0.2 (6.5; -9-10) | -2 (6.5; -16-8) | 0.51 |
| | Mean (95% CI), adjusted‡ | 1.3 (-1.9-4.5) | 0.1 (-3.0-3.3) | -2.1 (-5.8-1.7) | 0.40 |

Table 23: Difference in results of hypoxic challenge between supine and prone position according to group. (Value in supine position minus value in prone position)

*Denotes that all means are geometric means due to skewness of the distribution.

†SD on natural scale.

‡Denotes marginal means adjusted for birth weight, gestational age, and postnatal age (set to mean values).

## 4.3 Discussion

There was a significantly greater rate of decline in minute ventilation during the hypoxic challenge in the prone position in infants of mothers who had misused substances in pregnancy compared to those whose mothers had smoked and the controls. No such difference was seen in the supine position. The results are supported by the findings of Martin et al. demonstrating that chronic intermittent hypoxia in piglets increased the magnitude of this hypoxic decline. The initial increase in ventilation in response to hypoxia is thought to be primarily mediated via peripheral chemoreceptors.(Ohtake et al., 1998) In contrast the decline in ventilation in response to prolonged hypoxia is centrally mediated.(Dawes et al., 1983) The results, therefore, suggesting that maternal substance misuse had a central effect on respiratory control. Maternal smoking (Morrow et al., 1988) and cocaine (Woods et al., 1987) use may alter placental blood flow inducing intermittent hypoxia which may account for the more marked decline phase of the response to hypoxia. Maternal opiate use may cause intermittent hypoxia via direct effects on maternal respiratory control.(Santiago et al., 1977) Fourteen adult methadone users were studied while breathing air, and during hypoxic and hypercarbic challenges. Both ventilatory responses were damped, and while breathing air frequent oxygen desaturation and hypercarbia occurred following methadone administration.(Santiago et al., 1977)

Ali et al. reported a significantly greater initial ventilatory increase in response to hypoxia in infants on substance misusing mothers compared to controls and infants of smoking mothers studied in the supine position.(Ali et al., 2016) Furthermore they found the hypoxic rate of decline was greater in infants of substance misusing mothers and smoking mothers compared to controls. In this study, the decline in ventilation in response to hypoxia was significantly greater in infants of substance misusing mothers compared to controls and infants of smoking mothers, but only in the prone position.

It is possible that the infants assessed in the present study had less exposure to either maternal smoking or substance misuse. In the study by Ali et al. there was a significant difference in birth weight and gestation between the infants of substance misusing, smoking

and control parents that was not seen in this cohort. While the control and infants of smoking mothers were of similar gestation and birth weight between the two studies, the infants of substance misusing mothers were born at a median gestation two weeks less in the study by Ali et al. compared to this study. When, however, birth weight centiles were compared between this study and that of Ali et al. there were no significant differences. A comparison of urinary cotinine levels from Ali et al and this study demonstrated significantly higher cotinine levels in Ali's study (median 145ng/ml range (11-8760) vs 130ng/ml (0-3240) p=0.036).. Furthermore, 9 of 21 infants of substance misusing mothers in the study by Ali et al. went on to require treatment for neonatal abstinence compared to 4 of 17 infants in this study.

Galland et al. studied the ventilatory response to a mixed asphyxia gas in infants in the prone and supine position, and in keeping with the present findings, demonstrated no difference in the response between the prone and supine position in the neonatal period.(Galland et al., 2000) When, however, studied at three months of age the prone position was associated with a damped response. The authors proposed that the additional weight of a three month old compared to a newborn imparted a mechanical disadvantage in the prone position.

Lewis et al. studied the ventilatory response to hypoxia in infants of smoking mothers and unexposed controls between two and three months of age and found no difference in the response. These infants were placed in their usual sleeping position, either prone or supine, and the effect of position on response was not considered.(Lewis and Bosque, 1995)

This study had strengths and some limitations. To my knowledge this is the first study to evaluate the combined effect of sleeping position with the antenatal risk factors for SIDS: maternal smoking and substance misuse. The assessment of the hypoxic response quantified both the initial hypoxic ventilatory response and the later decline in ventilation,. The latter component has frequently been ignored in the assessment of hypoxic responses,(Lewis and Bosque, 1995) despite persisting at the high-risk age for SIDS. (Cohen et al., 1997) I did not rely on maternal reporting of smoking and substance misuse in order to determine the group to which an infant belonged, but utilised maternal and infant urine screens, along with maternal reporting to objectively assess exposure. This study has limitations. Unfortunately I did not achieve my target recruitment for the substance misuse group meaning the study may

have been underpowered to detect an effect of substance misuse on the ventilatory response to hypoxia. Measurements were performed in the neonatal period, at a low risk period for SIDS, and the effect of both sleep position and maternal substance misuse and smoking on ventilatory responses may only become apparent towards the high risk period.(Galland et al., 2000)

The effect of increasing ventilation in response to hypoxia is a concomitant reduction in end-tidal $CO_2$. As the response to hypoxia is modulated by $CO_2$ levels (Brady and Dunn, 1970) ideally the hypoxic challenge would have been conducted under isocapnic conditions, with additional inspired $CO_2$ maintaining a constant end-tidal $CO_2$ throughout the study. While this technique has been used in adults, (Rispen et al., 2017, Battisti-Charbonney et al., 2011, Schiffman et al., 1982) I am not aware that this technique has been employed in infants, and this was not technically feasible in our study due to natural fluctuations in end-tidal $CO_2$ such that a stable level could not be obtained through supplementation. As isocapnic hypoxia could not be achieved it is not possible to conclude that differences detected in the response to hypoxia are entirely independent of $CO_2$ mediated changes.

In conclusion, infants of substance misusing mothers in the prone position had a significantly more rapid decline in ventilation in response to hypoxia than infants exposed to only smoking or controls. This would impair the infant's ability to respond effectively to an exogenous stressor and could increase vulnerability to SIDS.

# Chapter 5 : The effect of caffeine on the ventilatory response to hypercarbia in preterm infants

Apnoea of prematurity is a common problem in prematurely born infants (Abu-Shaweesh and Martin, 2008) occurring most often in those born very prematurely.(Henderson-Smart, 1981) It is possible that a reduced chemoresponse to hypercarbia may contribute to the development of apnoea (Abu-Shaweesh and Martin, 2008) as chemosensitivity to carbon dioxide is a major contributor to respiratory drive in the preterm infant (Abu-Shaweesh and Martin, 2008).

Caffeine, a methylxanthine is commonly given to treat apnoea. Caffeine has been shown to increase the ventilatory response to hypercarbia. In anaesthetised cats caffeine administration resulted in an increase in minute ventilation, and an increase in the ventilatory response to inspired $CO_2$.(Mazzarelli et al., 1986)In a crossover study in which six adult males underwent a hypercapnic challenge prior to and following administration of intravenous caffeine, an enhanced ventilatory response to hypercarbia was demonstrated following caffeine administration.(Pianosi et al., 1994) In a further study of seven healthy adults an increased ventilatory response to hypercarbia following caffeine administration was also demonstrated.(D'Urzo et al., 1990)

Administration of theophylline, another methylxanthine, to ten infants with a median gestational age of 30 weeks and apnoea was associated with an increase in minute volume in response to breathing 3% $CO_2$ for five minutes and lower end-tidal $CO_2$ levels. (Davi et al., 1978) The measurements, however, were made prior to, and between two and four days after administration of theophylline, and no attempt was made to account for the possible maturation of the response to hypercarbia between the two measurements. (Davi et al., 1978) The effect of caffeine on the ventilatory response to hypercarbia has not been studied in prematurely born infants. In this study I tested the hypothesis that the ventilatory response to hypercarbia would be increased following administration of caffeine and the effect would be additional to the known positive effect of increasing maturation. (Rigatto et al., 1975) I further hypothesised that the ventilatory response to hypercarbia would reduce after caffeine was discontinued.

Furthermore, I hypothesized that a lower carbon dioxide sensitivity in asymptomatic infants would predict those infants who would go on to have significant apnoea, that is required treatment with caffeine.

## 5.1 Methods

A longitudinal study was performed at King's College Hospital NHS Foundation Trust between May 2013 and August 2015. Infants were eligible for entry into the study if they were born at less than 34 weeks of gestation, had no significant congenital abnormalities, did not require respiratory support or had received caffeine and were less than 72 hours of age. A hypercapnic challenge was undertaken and repeated weekly until discharge from the neonatal unit. Infants who developed significant apnoea (see later) during the course of the study were treated with caffeine. Parent(s) gave informed, written consent and the study was approved by the London Bromley Research Ethics Committee.

### 5.1.1 The hypercapnic challenge

The hypercapnic challenge was delivered via a nasal mask and pneumotachograph, using the equipment previously described (Chapter 2). Minute ventilation was measured during exposure to three levels of $CO_2$ (0% (baseline), 2% and 4%). Those levels were chosen as they have been previously demonstrated to result in changes in respiration without significant behavioural arousal.(Rigatto et al., 1975, Ali et al., 2014, Saiki et al., 2014, Smith et al., 2010) Each mixture of $CO_2$ was titrated using a low flow flow meter attached to the $CO_2$ regulator. When a stable inspiratory $CO_2$ concentration was achieved within the delivery tubing was determined from the capnograph readout. The infant breathed the air/$CO_2$ mixture for at least five minutes to allow ventilation and $ETCO_2$ to reach a stable state as assessed from the real-time display using the Spectra software (Grove Medical, London, UK). This duration is in keeping with previous studies. (Rigatto et al., 1975)The order of administration of the test gases was randomised for each infant.

Breath by breath data were exported from Spectra software to Microsoft Excel 2011 (Microsoft). Minute volume was calculated from the last minute of exposure at each level of $CO_2$. $CO_2$ sensitivity was calculated by the gradient of a line of best fit through a plot of the minute ventilations against the inspired $CO_2$ levels.

### 5.1.2 Polysomnography

Polysomnography was performed at the initial study to determine whether the infant was having apnoeas. A commercially available Alice 4 sleep study unit (Profile Vio-systems, Bognor Regis, UK) using the Alice 5 firmware upgrade was used. Abdominal and thoracic movements were assessed using stretch sensitive piezo-electric respiratory bands. Oral and nasal airflow was measured using the analogue output of the pneumotachograph described above. An electrocardiogram was recorded using single use bipolar electrodes. Two activity meters were attached one to an arm and the other to a leg to record limb movements. Oxygen saturation was continuously monitored using a pulse oximeter (Massimo rainbow SET Pulse Oximetry), the data were incorporated into the Alice sleep system using an auxiliary input.

The Alice sleep system was connected to a PC that was used to display the recording in real time and store data. The infant was monitored by video camera throughout the study with recordings stored on the PC. These recordings were used to assess sleep state and evaluate for artefact, such as awakenings or handling of the infant. Apnoeas were defined as cessation of respiratory airflow of five seconds.(Bhat et al., 2006) Apnoeas were classified as obstructive if there was no airflow despite chest and abdominal wall movements; central if there was no nasal airflow and an absence of chest and abdominal wall movements; and mixed if there was a combination of central and obstructive apnoeas. For each apnoea associated changes in heart rate and oxygen saturations were recorded.

The apnoea index (frequency of apnoeas per hour) was calculated for each infant at their first study.

### 5.1.3 Caffeine administration

The decision to treat with caffeine was made by the lead clinician without knowledge of the results of the polysomnography or hypercarbic challenge. The criteria for treatment with caffeine was significant apnoea which was defined as an apnoea lasting more than twenty seconds, or more than 10 seconds if associated with oxygen desaturation to <90% or bradycardia.(Finer et al., 2006) A loading dose of 20mg/kg caffeine citrate was administered intravenously, followed by maintenance dose of 10mg/kg every 24 hours given either

intravenously or enterally. Caffeine therapy was discontinued at 34 weeks corrected gestational age, or earlier if the infant had been symptom free for at least one week.

### 5.1.4 Data collection

Birth weight, gestational age at birth, maternal age and ethnicity were recorded. The infants were considered to have received antenatal steroids if at least one dose of steroids was given at least 24 hours prior to delivery. A diagnosis of chorioamnionitis was made if documented by the obstetricians, or if the mother received parenteral antibiotics because of clinical features of chorioamnionitis (maternal fever, fetal tachycardia or offensive liquor). At each study the current weight, corrected gestational age and postnatal age and caffeine treatment were recorded from the medical records.

### 5.1.5 Sample size

A sample of 30 infants would give a stable estimate of the standard deviation of the rate of change of $CO_2$ sensitivity. It was assumed that two-thirds of the infants would develop significant apnoea.(Henderson-Smart, 1981) Thus from the sample it was expected that it would be possible to analyse $CO_2$ sensitivity pre and post-caffeine in 17 infants, which would allow detection of a difference of one standard deviation with 80% power and 5% significance level.

### 5.1.6 Statistical analysis

Results were assessed for normal distribution visually using histograms and confirmed using the Shapiro-Wilks test and found to be normally distributed. Differences in outcomes were assessed for statistical significance using Fisher's exact test or the student t-test as appropriate.

Linear mixed models with fixed effects were developed to account for repeated measures, with carbon dioxide sensitivity as the dependent variable. Corrected gestational age was entered as a covariate, with caffeine therapy as a factor.

To determine predictors of apnoea, gestational age at birth, birth weight and $CO_2$ sensitivity were entered into a binary logistic regression model with the dichotomous dependent variable being a requirement for caffeine therapy. Probabilities from the binary logistic regression models were used to produce receiver operator characteristic (ROC) curves for predictors of apnoea. Analyses were conducted using SPSS 22 (IBM).

## 5.2 Results

Twenty-six infants born at a median gestation of 32 (range 31-33) weeks and a median birth weight 1590 (range 840 – 2200) grams were recruited into the study. They were first studied at a median 22 (range 6-69) hours after birth. Ninety-two studies were performed with a median of 4 (range 1-6) studies per infants. Fourteen infants developed significant apnoea and were treated with caffeine (Table 24).

| | No apnoea | Apnoea | p-value |
|---|---|---|---|
| n | 12 | 14 | |
| Birth weight (g) | 1835 (1120-2200) | 1545 (840-1840) | 0.03 |
| Gestational age (weeks) | 32+4 (32-33+5) | 32+1 (31+1-33+5) | 0.05 |
| Maternal age (years) | 35 (20-44) | 29 (18-38) | 0.45 |
| Race: | | | |
| White | 4/12 | 6/14 | 0.70 |
| Black | 6/12 | 5/14 | 0.69 |
| Asian | 1/12 | 1/14 | 1.00 |
| Other | 1/12 | 2/14 | 1.00 |
| Antenatal steroids | 10/12 | 13/14 | 0.58 |
| Chorioamnionitis | 1/12 | 3/14 | 0.60 |
| Caesarean section | 10/12 | 10/14 | 0.65 |
| Sex (F) | 6/12 | 5/14 | 0.69 |
| Birth weight <10th centile | 3/12 | 6/14 | 0.43 |
| Singleton | 10/12 | 9/14 | 0.39 |
| Apgar at 5 minutes | 10 (9-10) | 9 (8-10) | 0.37 |
| Supplementary oxygen >12 hours | 7/12 | 7/14 | 0.71 |
| Positive pressure support >12 hours | 3/12 | 5/14 | 0.68 |

Table 24: Demographics of study population according to significant apnoea. All data presented as median (range)

Carbon dioxide sensitivity was significantly higher following caffeine administration (p=0.006) (Figure 19).

Carbon dioxide sensitivity was significantly lower after discontinuing caffeine therapy than when measured prior to discontinuation (p=0.004). (Figure 20)

A linear mixed model of results from infants who did not receive caffeine demonstrated that corrected gestational age was associated with an increase in carbon dioxide sensitivity. One week increase in corrected gestational age correlated with an increase in carbon dioxide sensitivity of 9.5ml/kg/min/%$CO_2$ (95% CI: 4.3-14.6; p<0.001). When caffeine was introduced as a factor, and all infants included, corrected gestational age and caffeine both significantly contributed to a linear mixed model for $CO_2$ sensitivity. The estimated effect of corrected gestational age was an increase of 10.0ml/kg/min/%$CO_2$ (95% CI: 5.4-14.5) per week. The estimated marginal mean effect of caffeine therapy on $CO_2$ sensitivity at 33 weeks gestation was 15.3 ml/kg/min/%$CO_2$; (95% CI: 1-30).

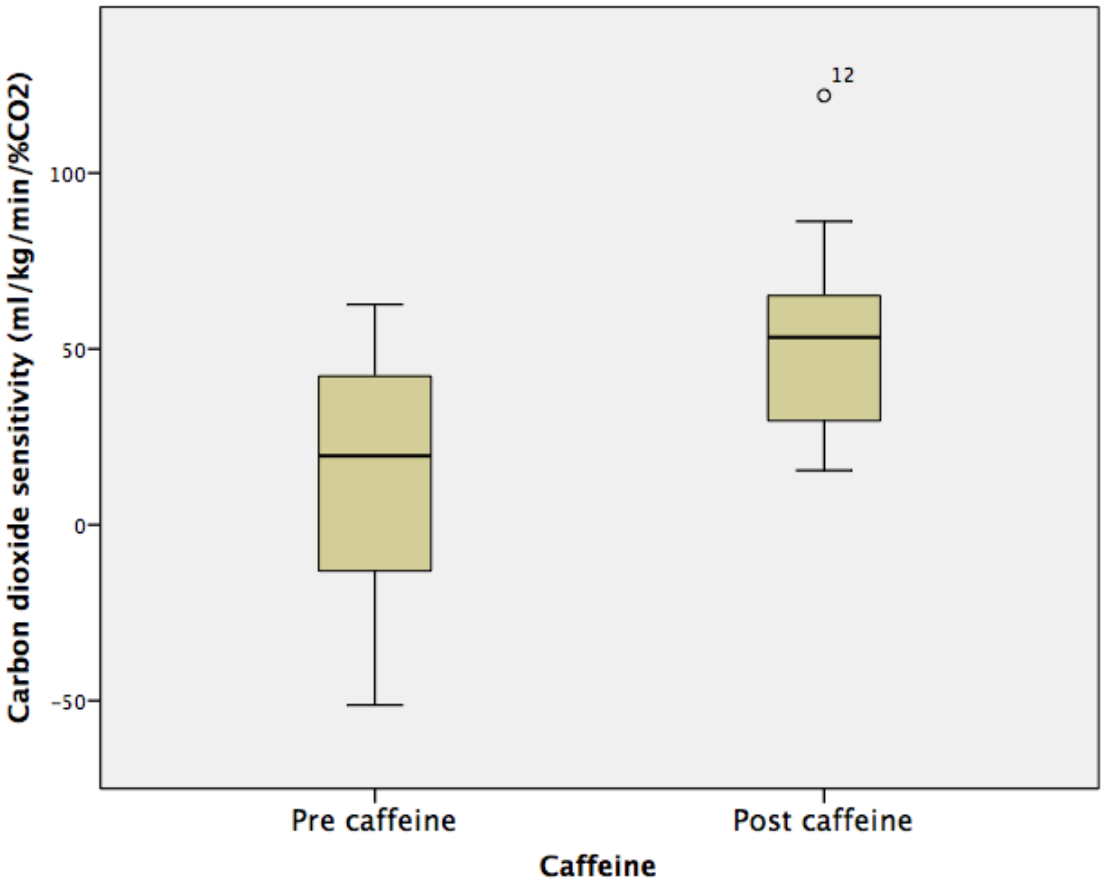


Figure 19: Box plot showing carbon dioxide sensitivity before and after administration of caffeine. . Box plot shows median and interquartile range. Whiskers extend to 1.5 times the interquartile range. Data points beyond this are marked as outliers.

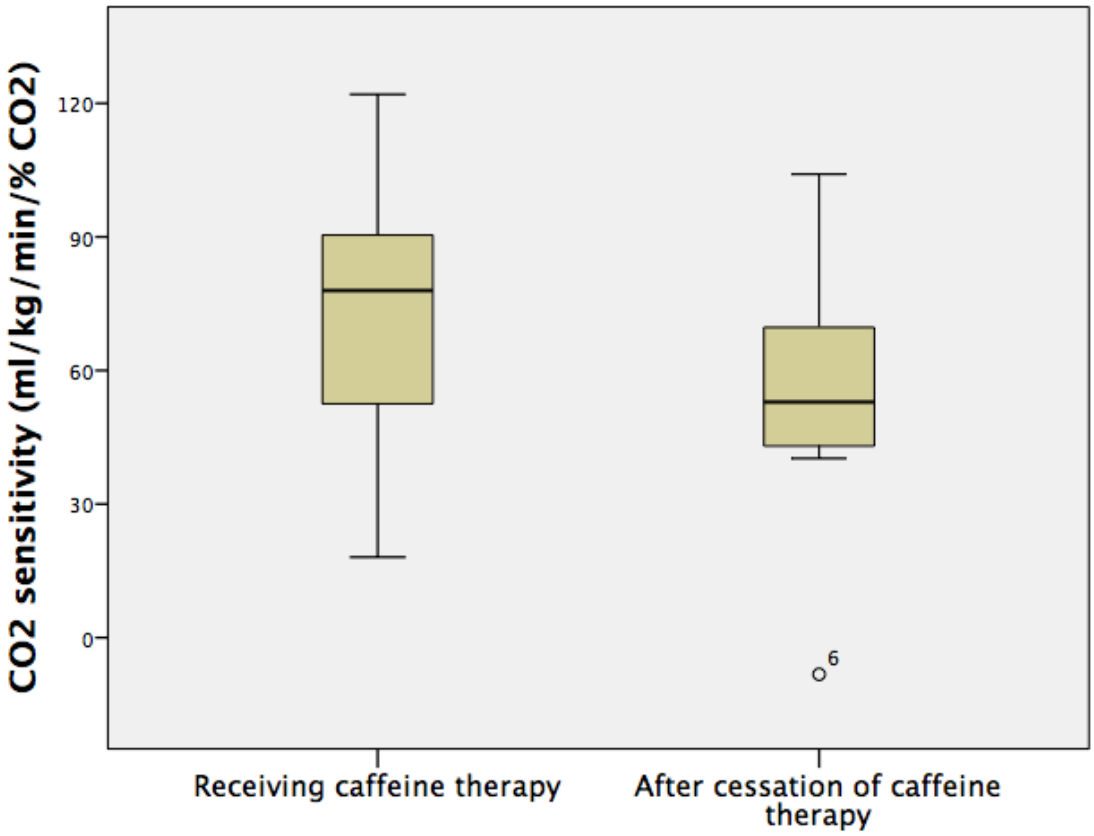


Figure 20: Box plot showing carbon dioxide sensitivity while receiving caffeine therapy, and carbon dioxide sensitivity at the following measurement one week later, following cessation of caffeine therapy.

At the initial study, there was no difference in the apnoea index between those infants that would go on to develop significant apnoea, and those that would not. Infants who developed apnoea had significantly lower birth weights and had a trend towards a lower gestational age and lower ventilatory response to carbon dioxide than those who did not require treatment (Table 25).

The predicted risk factors for development of apnoea (lower birth weight (1545g (840-1840) vs 1835g (1120-2200); p=0.03), lower gestational age (32+1 weeks (31+1-33+5) vs 32+4 weeks (32-33+5); p=0.04) , and lower carbon dioxide sensitivity (13ml/kg/%$CO_2$ (36) vs 34ml/kg/%$CO_2$ (15); p=0.065) ) all differed between the apnoea and non-apnoea group at a significance level of p<0.1 and were therefore entered into a binary logistic regression model.

When birth weight, gestational age and $CO_2$ sensitivity were entered into a binary logistic regression model, $CO_2$ sensitivity remained a predictor of significant apnoea. An increase in $CO_2$ sensitivity of 1 ml/kg/min/%$CO_2$ reduced the odds of developing significant apnoea by 6% (p=0.049).

The area under a receiver operator characteristic (AUROC) curve for $CO_2$ sensitivity, however, was 0.64 (95% CI 0.42-0.87), which was not statistically significant. A carbon dioxide sensitivity of less than 14ml/kg/min/%$CO_2$ had a sensitivity of 92% and specificity of 50% in predicting the development of apnoea requiring treatment.

Using the results of the logistic regression model incorporating gestational age, birth weight and $CO_2$ sensitivity the AUROC curve was 0.89 (95% CI 0.75-1; p=0.001).

| | No apnoea | Apnoea | p-value |
|---|---|---|---|
| Baseline minute volume (ml/kg/min) | 431 (91) | 426 (165) | 0.920 |
| Baseline end-tidal $CO_2$ (%) | 4.1 (0.7) | 4.4 (0.8) | 0.222 |
| Apnoea index (events/hr) | 3.8 (3.6) | 3.1 (2.4) | 0.608 |
| $CO_2$ sensitivity (ml/kg/min/%$CO_2$) | 34 (15) | 13 (36) | 0.065 |

Table 25: Results of first study, grouped according to whether the infant went on to develop significant apnoea requiring caffeine therapy. Data are presented as mean (SD)

| | AUROC curve | 95% confidence interval | p-value |
|---|---|---|---|
| Birth weight | 0.738 | 0.530-0.946 | 0.04 |
| Gestational age | 0.750 | 0.558-0.942 | 0.031 |
| $CO_2$ sensitivity | 0.643 | 0.418-0.868 | 0.217 |
| Birth weight and gestational age | 0.798 | 0.621-0.974 | 0.01 |
| Birth weight and $CO_2$ sensitivity | 0.821 | 0.654-0.989 | 0.005 |
| Gestational age and $CO_2$ sensitivity | 0.815 | 0.652-0.979 | 0.006 |
| Birth weight, gestational age and $CO_2$ sensitivity | 0.887 | 0.752-1.00 | 0.001 |

Table 26: Area under receiver operator characteristic curves for binary logistic regression models incorporating combinations of predictors

## 5.3 Discussion

This study has demonstrated that caffeine administration is associated with an increase in the ventilatory response to hypercarbia in prematurely born infants and that this effect remained significant after controlling for increasing maturity. When caffeine was discontinued, $CO_2$ sensitivity significantly decreased, suggesting that the effect of caffeine is not a permanent modulation of $CO_2$ sensitivity. No infant developed further apnoea after cessation of therapy, suggesting that the intrinsic chemosensitivity of the infant had increased to a level at which significant apnoea was less likely.

In addition, a poorer response to the initial hypercarbic challenge was commoner in those who went on to develop significant apnoea.

Those findings are consistent with the reports that a weaker response to hypercarbia has been found in infants with significant apnoea. Gerhardt and Bancalari measured the ventilatory response to $CO_2$ in 18 infants with significant apnoea (a mean of 32 episodes a day of apnoea lasting longer than 20 seconds) and 18 matched controls without apnoea. At approximately a week of age those with apnoea had a reduced ventilatory response to $CO_2$.(Gerhardt and Bancalari, 1984)

Importantly, our study has demonstrated that reduced carbon dioxide sensitivity in apnoeic infants precedes the development of apnoea, and is therefore more likely to be a contributive factor rather than a result of recurrent apnoea induced episodes of hypercarbia and hypoxia. Durand et al. compared eight preterm infants with at least three episodes a day of apnoea lasting more than 20 seconds to nine preterm infants without apnoea and reported reduced baseline ventilation and $CO_2$ sensitivity in infants with apnoea.(Durand et al., 1985) However, these findings could plausibly be a result of chronic intermittent hypoxia due to apnoea, rather than be a cause.

Intermittent hypoxia may modulate the subsequent ventilatory responses to hypercarbia.(Kinkead et al., 2001) Exposure of neonatal piglets to intermittent hypercapnic and hypoxia reduced the subsequent ventilatory response to hypercarbia.(Waters and Tinworth, 2001) Two studies, however, in rat pups using several different regimes of hypercapnic or normocapnic hypoxia failed to demonstrate a significant effect on the subsequent ventilatory response to hypercarbia.(Millstrom et al., 2015, Peng and Prabhakar, 2004) Millstrom exposed newborn rat pups to regimes of intermittent hypercapnic hypoxia (6% $CO_2$/10% $O_2$), or intermittent hypercapnic hypoxia alternating with hyperoxia (30% $O_2$), five times an hour over the first two weeks after birth. Neither regime had any subsequent effect on the ventilatory response to hypercarbia.(Millstrom et al., 2015) Peng used a shorter duration of intermittent hypoxia (15 seconds of 5% $O_2$, nine times an hour for eight hours a day during the first ten days after birth in newborn rats, and again showed no effect on the hypercapnic response compared to non-exposed controls.(Peng and Prabhakar, 2004) In this study examining infants prior to development of significant apnoea, we suggest that a damped ventilatory response to hypercarbia contributes to the development of apnoea, rather than being a result of intermittent hypoxia during apnoeic periods.

This study contributes to the literature that administration of caffeine is beneficial for prematurely born infants. Methylxanthines have been shown to reduce the frequency of apnoeas in preterm infants. (Henderson-Smart and Steer, 2001)Methylxanthines are phosphodiesterase inhibitors and at therapeutic concentrations, are non-specific adenosine receptor antagonists. Adenosine receptors are expressed throughout the brain stem respiratory centres. There are several subtypes, blockade of which have varying effects on respiratory control.(Bianchi and Gestreau, 2009, Martin et al., 2004, Fredholm, 1995) Adenosine $A_{2A}$ receptors play a role in the development of late hypoxic ventilatory depression, while $A_1$ receptors contribute to cardiorespiratory control during normoxia.(Simakajornboon and Kuptanon, 2005) The role of these receptors changes with development. Elnezir demonstrated that adenosine receptor antagonists blocked late hypoxic ventilatory decline in three day old piglets, but had no effect at three weeks postnatal age.(Elnazir et al., 1996) Polymorphisms of genes encoding Adenosine A1 receptors may contribute to the development of apnoea of prematurity, and the response to caffeine therapy. Cord blood was taken from 115 infants born between 28 and 34 weeks gestation, and analysed for six single

nucleotide polymorphisms (SNPs) of the genes encoding Adenosine A1 and $2_A$ receptors. Three of the Adenosine $A2_A$ receptor SNPs were associated with apnoea development. One Adenosine A1 receptor SNP was associated with a positive therapeutic response to caffeine therapy, but only in infants over 28 weeks gestation. (Kumral et al., 2012)

Use of caffeine is associated with long term benefits. In a large randomised study treatment of apnoea of prematurity in low birth weight infants (500-1250g) with caffeine rather than placebo was associated with a shorter duration of positive pressure ventilation, reduced rates of bronchopulmonary dysplasia (Schmidt et al., 2006) and better neurodevelopmental outcomes at 18 to 21 months of age.(Schmidt et al., 2007) Indications for administering caffeine in that study were treatment of apnoea, prevention of apnoea or facilitation of removal of the endotracheal tube. The differences in neurodevelopmental outcomes were, however, no longer statistically significant when the children were re-examined at five years of age.(Schmidt et al., 2012)

This study has strengths and some limitations. A strength of our study is the longitudinal measurement of infants, including those that did not require caffeine treatment, which has allowed control for the maturational effect on the ventilatory response to hypercarbia.

Our study required recruitment of infants born sufficiently prematurely that they were likely to develop apnoea, but did not require respiratory support. This, then, was a highly selective group which presented difficulties in recruiting to the targeted sample size. A smaller proportion of eligible infants went on to require treatment with caffeine than anticipated in the sample size calculation. Nevertheless, significant differences were seen.

In conclusion, caffeine administration was associated with an increase in the ventilatory response to hypercarbia. Furthermore, a lower ventilatory response to carbon dioxide in healthy newborn infants predicted those infants that will go on to require treatment for significant apnoea.

# Chapter 6 : Investigation and management of gastro-oesophageal reflux in neonatal intensive care units in the United Kingdom

Gastro-oesophageal reflux (GOR), the retrograde movement of gastric contents into the oesophagus, is a common event even in healthy infants.(Vandenplas et al., 2005) Gastro-oesophageal reflux disease (GORD) occurs when the reflux causes troublesome symptoms. The symptoms attributed to GORD, however, are varied and non-specific and include that the infants is unsettled, has poor weight gain, back arching, poor feeding and/or respiratory disturbance, including apnoea. Many infants are treated for GORD, for example approximately 25% of 1598 extremely low birth weight (<1000g) infants were discharged from neonatal intensive care units (NICUs) in the USA on anti-reflux medication.(Malcolm et al., 2008) In 2004, a survey was reported of the diagnostic and treatment strategies for GORD in major NICUs in the United Kingdom.(Dhillon and Ewer, 2004) A wide variation in strategies was demonstrated, which the authors postulated resulted from the lack of published evidence.(Dhillon and Ewer, 2004) The aim of this study was to conduct a further survey to determine current diagnostic and treatment strategies and if practice now reflected an increased evidence base.

## 6.1 Methods

A questionnaire was sent to the lead clinicians of all 207 UK neonatal units identified from the National Neonatal Audit Programm,the British Association of Perinatal Medicine directory and a departmental database established for previous audits. (Appendix A) Practitioners were asked which investigations were used, what thresholds were considered abnormal for pH and multichannel intraluminal impendence (MII) studies and what proportion of infants were investigated prior to initiation of therapy for GORD. In addition, they were asked which medications were used as first line treatment and what criteria were used for discontinuing treatment.

## 6.2 Results

Responses were obtained from 84% of the 207 UK neonatal units. To establish the diagnosis, the majority (58%) of units used a trial of treatment; pH studies were used in 24% of units, GI contrast studies in 23%, MII/pH in 6%, abdominal ultrasounds in 3% and gastro-oesophageal scintigraphy in 2%. Only six units suggested a threshold for an abnormal pH study. An abnormal acid index of more than 5% was considered abnormal in one unit, more than 7% in two units and more than 10% in three units. Only two units gave a threshold for an abnormal MII study and in both, this was more than 50 reflux events in 24 hours. In only two units was anti-reflux medication never started prior to investigation and in 32% of units medication was always started without investigation. In a further 29% of units, treatment was often started without investigation and in another 19% of units occasionally without investigation.

The type of treatment used varied widely between neonatal units. The most commonly used medication was Gaviscon and this was in 72% of units. Other treatments included ranitidine (53% of units), thickening agents (27% of units) and proton pump inhibitors (23% of units). The prokinetics domperidone (22% of units) and erythromycin (6% of units) were also used. Therapy was discontinued in 44% units when symptoms resolved, in 9% of units prior to discharge and in 19% of units at the first out patient appointment. Three percent of units undertook a trial of withdrawing treatment and medication was discontinued if there was no clear benefit.

## 6.3 Discussion

We had demonstrated that there was a wide variation in the current investigation and management strategies for infants with suspected GORD being cared for on UK neonatal units. The previous survey (Dhillon and Ewer, 2004) also showed a similar wide variation, but was only of prematurely born infants cared in level two and three neonatal units. In our survey, all 207 neonatal units of all levels were included with an 84% response rate. In addition, we asked about practice for all infants, regardless of maturity at birth.

A trial of therapy was the most commonly used approach to diagnose GORD and surprisingly few neonatal units had access to pH or MII studies. In the 2004 survey only 30% of units used pH monitoring on a regular basis.(Dhillon and Ewer, 2004) The current survey revealed that still only a minority of units (34%) were using pH monitoring, perhaps reflecting the poor correlation between symptoms and the results of investigations (Corvaglia et al., 2013a) and/or the problems with interpreting the results. Reflux events are diagnosed if there is a reduction in the oesophageal pH below four.(Tuttle et al., 1961) The acid index is also used to diagnose reflux and is the percentage of time in 24 hours the oesophageal pH is less than four.(Newell et al., 1989) In infants it has been suggested that such criteria may be of limited use as the gastric contents can be buffered by milk (Grant and Cochran, 2001) and the contents have a pH of more than 4 for up to 90% of the time.(Grant and Cochran, 2001) The concerns regarding the buffering effect of milk, however, were based on data from studies with small numbers of heterogeneous groups of patients (Washington et al., 1999, Mitchell et al., 2001). Other studies have shown that gastric pH dispersion is not homogeneous and have failed to find a significant effect of gastric buffering on the reflux index (RI).(Badriul et al., 1999, Hegar et al., 2000) A reference range for pH studies was established from the results of 509 infants, but, although they were healthy at the time of investigation, they were all at high risk of sudden infant death syndrome.(Vandenplas et al., 1991) These limited data may explain why only six units gave criteria for an abnormal pH study and they used different values. A further problem with using pH monitoring to diagnose reflux events is non-acid reflux.(Wenzl et al., 2002) In contrast, MII monitoring can detect all reflux events, that is, both acid and non-acid reflux. The direction, height and duration of bolus movements can also be determined.(Wenzl

et al., 2002, Wenzl, 2002) In one study, there was no statistically significant correlation was found between the pH reflux index and the duration of treatment, but a significant correlation between MII results and the duration of treatment, although the correlation was low ($r^2$=0.36).(De Rose et al., 2014) In another study, 64 newborns with GORD symptoms who underwent MII/pH monitoring in the first weeks after birth and then sequentially over the next three years impedance bolus exposure index and proximal reflux frequency were most predictive of the duration of symptoms.(Cresi et al., 2013) Yet, we only found 5% of units used this technique. Only two units gave a threshold for an abnormal MII study, both used more than 50 events in 24 hours. A study of twenty-one healthy, preterm infants, however, suggested that up to one hundred reflux events in 24 hours was normal.(Vandenplas and Loeb, 1990) Upper GI contrast studies were used in 23% of units to diagnose GORD and, although they have a role in investigating anatomical abnormalities, they cannot quantify the frequency of reflux events and hence determine the severity.(Vandenplas et al., 2009)

There was a wide variety of management strategies for GORD, as was demonstrated previously.(Dhillon and Ewer, 2004) Gaviscon was the most commonly used treatment, perhaps reflecting it has minimal side-effects.(Del Buono et al., 2005) Gaviscon, however, is contra-indicated in those with known or suspected impairment of renal function and should not be given with other preparations that contain thickening agents. Other medications used include histamine 2 receptor blockers, such as ranitidine, and proton pump inhibitors which have been shown to be efficacious in reducing gastric acid secretion and increasing intra-gastric pH.(Kelly et al., 1993, Kelly, 1994) Both types of medication, however, have been associated with increased rates of necrotising enterocolitis and serious bacterial infections.(Orenstein et al., 2009) A systematic review which included one case control and one prospective cohort study demonstrated a significant association between NEC and inhibitors of gastric acid production, as well as a higher incidence of infection (sepsis and pneumonia).(More et al., 2013) Dopamine receptor antagonists such as the macrolides domperidone and erythromycin, have been shown to improve clinical symptoms,(De Loore et al., 1979, Carroccio et al., 1994) and pH study indices (Carroccio et al., 1994, Bines et al., 1992, Tolia et al., 1989), however systematic reviews evaluating the use of domperidone in the treatment of GORD in children (Pritchard et al., 2005) and infants (Scott, 2012) concluded that there was insufficient evidence to support its use. Domperidone can precipitate cardiac

arrhythmias,(Doggrell and Hancox, 2014) yet was currently used in 27% of UK neonatal units and erythromycin use has been associated with hypertrophic pyloric stenosis.(Lund et al., 2014) No unit was using cisapride reflecting that this medication may also precipitate arrhythmias.(Agency., 1998) In the 2004 survey, two thirds of units reported using cisapride to treat GORD,(Dhillon and Ewer, 2004) yet the survey was carried out a year after the Committee on Safety of Medicines report.(Agency., 1998) The majority of units (78%), however, performed an electrocardiogram before and after starting treatment.(Dhillon and Ewer, 2004) Thickening agents were currently used in 27% of units. Although some studies (Vandenplas et al., 1994, Orenstein et al., 1987) have demonstrated benefit from thickening agents this is not a consistent finding.(Bailey et al., 1987, Forbes et al., 1986) A study of 24 prematurely born infants demonstrated thickening a formula with amylopectin did reduce acid GOR, but not non acid GOR indexes or GOR related apnoeas.(Corvaglia et al., 2013b) A survey of neonatal feeding therapists and providers demonstrated the majority were using thickened feeds for dysphagia or GERD, but noted variability in prescriptions for thickening agents regarding consistencies, thickening agents and recipes.(Madhoun et al., 2015)

In conclusion, we have demonstrated there is no consistency regarding investigation or medication use in infants with suspected GORD in neonatal units in the UK. As in 2004, this likely reflects a limited evidence base and highlights the need for appropriate studies to inform best practice.

# Chapter 7 : Detection of gastro-oesophageal reflux on the neonatal unit

Gastro-oesophageal reflux (GOR) is a frequent phenomenon in infants and when associated with morbidity is called gastro-oesophageal reflux disease (GORD). Infants, suspected of GORD should undergo appropriate investigations. In infants, however, association between symptoms and the results of pH or endoscopy studies is weak.(Orenstein et al., 1996, Aggarwal et al., 2004, Salvatore et al., 2005) Furthermore, a reflux event is diagnosed if the pH is less than four (Boix-Ochoa et al., 1980, Tuttle et al., 1961), yet prematurely born infants may have a gastric pH of >4 for up to 90% of the time (Grant and Cochran, 2001) and non acid reflux may also cause symptoms. Multichannel intraluminal impedance (MII) detects all oesophageal bolus movements irrespective of changes in pH. A combination of monitoring with MII and a pH probe would seem likely to result in greater detection of reflux events, but data are conflicting.(Grant and Cochran, 2001, Condino et al., 2006, Blasco-Alonso et al., 2014, Woodley and Mousa, 2006, Corvaglia et al., 2009, Di Fiore et al., 2009) While these studies demonstrated a number of non-acid events undetected by pH study alone, the number of acid reflux events also detected by MII varied greatly. This raises the question of the clinical significance of differently derived reflux parameters. Intraluminal impedance allowed detection of bolus movement, but may also provide a measure of mucosal integrity, with damaged mucosa having lower baseline intraluminal impedance than healthy mucosa.(Loots et al., 2012)

Our aim, therefore, was to use combined pH/MII monitoring in a neonatal intensive care unit (NICU) setting to determine what proportion of infants with clinically suspected GORD had GORD. In addition, we wished to determine, as in children,(van der Pol et al., 2013) whether the results of MII monitoring related to baseline oesophageal impedance.

## 7.1 Methods

Infants with suspected GORD were assessed as part of their routine clinical care between March 2013 and August 2015. Each infant underwent a minimum of 20 hours of continuous oesophageal pH and MII assessment. The equipment was as described in Chapter 2.

### 7.1.1 Protocol

The infant's length was measured and oesophageal length estimated according to Strobel's formula for infants over 40cm in length (Strobel et al., 1979) and by a nomogram for those under 40cm (Omari et al., 1999). The probe was inserted through a nostril and secured at the required length. A chest radiograph was then obtained to determine if the pH sensor was appropriately positioned between the sixth and eighth thoracic vertebra. The position of the probe at the nares was reassessed following completion of the study to ensure the probe had not been displaced.

Following confirmation of the probe position, recording was commenced. The Zephyr Sleuth system (Sandhill Scientific) continuously recorded impedance and pH data with a sampling frequency of 50 Hertz.

Analysis of the traces produced was performed using Bioview Analysis software (Sandhill Scientific) and by manual review of the traces. Reflux parameters were derived as described in Chapter 2.

The acid index was the total time when the oesophageal pH was less than four calculated as a percentage of the total study time. GORD was diagnosed if the acid index was greater than 10%.(Rudolph et al., 2001)

MII reflux events were diagnosed when there was a drop in impedance to less than 50% of the baseline at the most distal channel, which moved retrogradely across at least two channels. These were further classified as acid (pH <4), weakly acid (4> pH <7) or alkali (pH >7). GORD was diagnosed if the number of impedance detected events was greater than 79, which was

the 90$^{th}$ centile in a study of forty-six healthy infants investigated for possible reflux related symptoms, who had no symptom association, and acid index of less than the median acid index value derived from previous studies.(Mousa et al., 2014) This was roughly equivalent to the 75$^{th}$ centile in a study of 20 asymptomatic, prematurely born infants.(Lopez-Alonso et al., 2006)

The baseline impedance was calculated from the most distal channel using an algorithm with the effect of gas and liquid boluses being excluded. (van der Pol et al., 2013)

To assess the reproducibility of MII scoring, two researchers independently scored ten randomly selected studies and the reliability measure based on intra-class correlation was calculated.

### 7.1.2 Sample size

As this was a retrospective review of studies no sample size calculation was undertaken.

### 7.1.3 Analysis

Four groups were defined and analysed according to the results of the pH and MII monitoring: group 1 – negative pH and negative MII; group 2-positive pH, negative MII; group 3 – negative pH, positive MII; group 4 – positive pH and positive MII. Differences between groups were assessed for statistical significance using the Kruskal-Wallis test. The strength of relations between baseline impedance and other reflux indices were determined by calculating Spearman correlation coefficients and were adjusted for postnatal and gestational age using partial rank correlation. The statistical analysis was performed using SPSS 22 (SPSS Inc, Chicago, IL).

## 7.2 Results

Forty-two infants (20 male) with a median gestational age of 31 (range 23-42) weeks and median birth weight of 1740 (range 550-3890) grams were assessed at a median post menstrual age (PMA) of 38 (range 30-60) weeks and postnatal age of 54 (range 2 -250) days. The infants were suspected of GORD because of desaturation with feeding (n=27), unexplained apnoea (n=6), poor feeding or oral aversion (n=3), poor weight gain (n=4) and an apparent life threatening event (n=2). A total of 1006 hours of combined pH and MII monitoring was undertaken. There were 1,717 pH events with a median acid index of 4.7 (range 0-28)% and the mean acid clearance time was 112 seconds (standard deviation 70 seconds); only 585 of the events were also detected by MII monitoring. MII detected 2041 reflux events; 585 (29%) were “acid” and detected by pH monitoring; 1387 (68%) were weakly acid and 69 (3%) alkali events. The reproducibility of MII scoring was good, with an intra-class correlation coefficient of 0.985 (95% Confidence Intervals (CI), 0.899-0.997). The median mean bolus clearance time was 18 (range 6-62) seconds and the median maximum bolus clearance time was 118 (range 16-1200) seconds.

Seven infants had an abnormal pH study only; one had an abnormal MII study only and one had both abnormal pH and MII studies. There were no significant differences in the gestational age, postnatal age or baseline impedance between the groups (Table 27).

There was an inverse relationship between the baseline impedance and the maximum acid clearance time (r=-0.45, p=0.003), acid index (r=-0.37, p=0.021), and total number of pH detected events /24 hours (r=-0.33, p=0.039). There was a positive correlation between postnatal age and baseline impedance (r=0.75, p <0.001) and a negative correlation between baseline impedance and gestational age (r=-0.54, p<0.001). After correcting for gestational age and postnatal age, baseline impedance remained significantly inversely correlated with the maximum acid clearance time (r=-0.44, p=0.006) and acid index (r=-0.34, p=0.038). Both the number and duration of MII detected weakly acid reflux events were positively correlated with baseline impedance (r=0.33, p=0.045; r=0.32, p=0.048 respectively).

| | pH – MII – n=33 | pH + MII – n=7 | pH – MII + n=1 | pH + MII + n=1 | p-value |
|---|---|---|---|---|---|
| **Gestational age (weeks)** | 29 (23-42) | 32 (27-39) | 36 | 32 | 0.61 |
| **Postnatal age (days)** | 56 (2-250) | 23 (4-73) | 18 | 31 | 0.48 |
| **Baseline oesophageal impedance(Ω)** | 1540 (300-2930) | 1510 (620-2000) | 1720 | 1290 | 0.71 |

Table 27: Characteristics grouped by investigation results. Data as median (range)

## 7.3 Discussion

We have demonstrated using combined pH/MII monitoring in an NICU setting, that despite a high index of clinical suspicion, fewer than 25% of studies were abnormal. Similar results have been found in studies which have investigated children and infants. In one study (Pilic et al., 2011) 39% had abnormal MII/pH studies and in another 4%.(Noviello et al., 2014) However, both these studies included a paediatric population. In a review of 58 MII/pH studies in symptomatic infants Funderburk et al. only 10% of studies were abnormal.(Funderburk et al., 2016) This study included both infants form the NICU, and older infants from outpatient clinic and paediatric wards.

Further investigation by pH study alone only 21% of infants suspected of having GORD had abnormal results.(Salvatore et al., 2005) A clinical diagnosis of GORD is, therefore, a poor predictor of abnormal MII or pH study. Our data emphasize the importance of investigating infants with suspected GORD and not empirically starting them on therapy. Indeed, medical management of GORD is not without risk. The use of a histamine blocker or proton pump inhibitor in the prematurely born population has been associated with an increased risk of necrotising enterocolitis and serious bacterial infection,(Terrin et al., 2012, Orenstein et al., 2009) and the prokinetic domperidone is associated with arrhythmias (Doggrell and Hancox, 2014).

Only 34% of pH detected events fulfilled the criteria for an MII reflux event. This low detection rate may reflect that a reflux bolus must cross a total of three bands to be registered as a reflux event and there could be acidification of the distal oesophagus which did not propagate far enough to be registered as a reflux event. In a study of 52 symptomatic prematurely born infants, it was shown that a greater number of acid reflux events were detected by pH rather than MII monitoring (3443 versus 672). (Corvaglia et al., 2009) In another study of 14 infants with median age 3.5 months, only 28% of pH detected acid reflux events were detected by MII.(Woodley and Mousa, 2006)

Baseline impedance after controlling for gestational and postnatal age was significantly negatively correlated with acid reflux events. Those results are supported by findings from 26 children, which demonstrated a negative correlation between reflux index and baseline impedance.(van der Pol et al., 2013) In addition, baseline impedance was demonstrated to increase following two weeks of proton pump inhibitor treatment in a randomised placebo controlled trial of 40 infants aged less than 6 months with symptomatic reflux.(Loots et al., 2012) Lower baseline impedance in adults has been correlated with non-erosive oesophagitis, oesophagitis, and pathological acid exposure.(Kessing et al., 2011) In addition, in adults it has also been associated with microscopic evidence of mucosal disruption and greater pain associated with reflux.(Barlow and Orlando, 2005) Lower baseline impedance may, therefore, reflect reflux associated mucosal damage. Our study is unique in evaluating the relationship between baseline impedance and other reflux parameters in a NICU population.

There are strengths and some limitations to our study. Our sample was heterogeneous with respect to gestational and postnatal age and symptoms, but reflects the population of infants cared on a neonatal intensive care unit and thus our results are generalisable. Although the infants were studied for clinical reasons, a standardised technique was used.
A comparison of infants by positive or negative pH or MII study is limited by the small number in each of these groups. Furthemore, as this was a retrospective review of clinical studies no sample size calculation was undertaken. Nevertheless the data presented are descriptive of a population in whom these techniques are widely used.

In conclusion, a clinical diagnosis of GORD is frequently inaccurate. Undertaking combined pH and MII monitoring improves the accuracy of detection of GORD. A low baseline oesophageal impedance may indicate disruption of mucosal integrity.

# Chapter 8 : Comparison of the diagnosis of gastro-oesophageal reflux in neonatal units by combined pH/multichannel intraluminal impedance studies and upper gastrointestinal contrast studies

In the investigation of gastro-oesophageal reflux, combined pH/MII studies can document information about the frequency and pH of reflux episodes. The pH study only detects acid reflux which may constitute a small proportion of reflux events in infants, whereas pH/MII studies detect the movement of small bolus volumes irrespective of acidity and allow prolonged assessment impossible with upper gastrointestinal contrast study (UGI).(Wenzl and Skopnik, 2000) MII/pH is, however, only available in a small minority (6%) of neonatal units in the United Kingdom.(Andradi et al., 2016) In a recent survey 23% of neonatal units in the United Kingdom used UGI to identify GOR.(Andradi et al., 2016) UGI will demonstrate reflux events irrespective of pH, but in the joint paediatric gastro-oesophageal reflux clinical guidelines by the European and North American societies for paediatric gastroenterology, hepatology and nutrition the use of UGI to diagnose GORD is discouraged as the brief window of assessment may miss clinically significant reflux events.

The aim of this study was to determine if there was any correlation between the results of positive pH/MII studies and upper GI contrast studies.

## 8.1 Methods

Data from infants who underwent pH/MII study and upper GI contrast study during the same admission were analysed providing they were receiving the same anti-reflux medication during both investigations. Each infant underwent a minimum of 20 hours of continuous oesophageal pH and MII assessment as previously described (Chapter 2). The acid index was the time the oesophageal pH was less than four calculated as a percentage of the total study time. GOR was diagnosed if the acid index was greater than 10%, the threshold for a positive study proposed in The ESPGHAN and NSPGHAN clinical guideline.(Vandenplas et al., 2009) The results were also analysed using a lower threshold of acid index greater than 5% as positive which has been used in a previous study.(Aksglaede et al., 2003) MII reflux events were diagnosed when there was a drop in impedance to less than 50% of the baseline at the most distal channel which moved retrogradely across at least two channels. GOR was diagnosed if the number of impedance detected events was greater than 79, which was the ninetieth percentile in a study of 42 healthy infants (Mousa et al., 2014) and above the fiftieth percentile in a study of 20 asymptomatic, prematurely born infants.(Lopez-Alonso et al., 2006) Upper GI contrast studies were reported by a consultant radiologist who performed the investigation. The presence of gastro-oesophageal reflux was determined by the height of the reflux column. A study was deemed positive if there was gastro-oesophageal reflux beyond the distal third of the oesophagus.(Aksglaede et al., 2003)

### 8.1.1 Sample size

This study aims to determine how sensitive UGI is in diagnosing GORD in a NICU population compared to pH/MII. Disease prevalence is estimated to be 20% based on the results presented in chapter 7. A minimum sample size of 155 infants (including 31 subjects with GORD) would be required to achieve a minimum power of 80% (actual power=80.7%) in order to detect a change in the percentage value of sensitivity from 0.70 to 0.90, based on a target significance level of 0.05 (actual p=0.048). This minimum sample size is also sufficient to detect a change in the value of specificity from 70.0% to 90.0% which will require a minimum sample of 39 infants (including 8 infants having GORD).

## 8.2 Results

Fifteen infants were included in the study (Table 28). Seven infants were receiving Domperidone and four also ranitidine during both the pH/MII and UGI studies.

Two of 15 pH/MII studies were positive by pH criteria (acid index >10%). Both had reflux to the proximal oesophagus on upper GI contrast study. None of the infants had an abnormal study according to MII criteria .Six infants had the UGI prior to the monitoring (median 9.5 range 1-20 days) and 9 after (median 5 range 0-63 days). In four studies no reflux was reported, in two studies reflux was reported to the distal third of the oesophagus. In the remaining nine studies reflux was reported above the distal third of the oesophagus or into the oropharynx.

The diagnosis of GOR by an upper GI contrast study compared to a pH/MII study had a sensitivity of 100% (95% CI 20-100%) with a specificity of 46% (95% CI 20-74%), using the higher acid index threshold (>10%) for a positive pH study.(Table 29)

Using the lower threshold of >5% for a positive pH study, the sensitivity was unchanged at 100% (95% CI 40-100%), with a specificity of 40% (95% CI 25-82%) (Table 30)

| | Median (range) |
|---|---|
| Gestational age at birth | 32 weeks (23-40) |
| Postnatal age at pH/MII study | 58 days (4-212) |
| Corrected gestational age at pH/MII study | 39 weeks (42-55) |
| Postnatal age at UGI study | 51 days (3– 231) |
| Corrected gestational age at UGI study | 44 weeks (39-57) |
| Acid Index at pH/MII study | 2.2% (0.1-29) |
| MII events/24 hours at pH/MII study | 8 (1-56) |

Table 28: Demographics and pH/MII results

| | UGI Positive | UGI Negative |
|---|---|---|
| MII/pH Positive | 2 | 0 |
| MII/pH Negative | 7 | 6 |

Table 29: Comparison of UGI and pH/MII positive and negative results using an acid index >10% as positive

| | UGI Positive | UGI Negative |
|---|---|---|
| MII/pH Positive | 4 | 0 |
| MII/pH Negative | 5 | 6 |

Table 30: Comparison of UGI and pH/MII positive and negative results using an acid index >5% as positive

## 8.3 Discussion

These results demonstrate a significant discrepancy between the results of pH/MII study and upper GI contrast studies, with more infants being diagnosed with GOR by positive upper GI contrast studies. The higher rate of UGI detection may be explained by the frequency of reflux events in healthy control infants. In a study of healthy, asymptomatic preterm infants using MII, a median of 71 reflux events occurred in 24 hours, of which 90% reached the proximal oesophagus.(Lopez-Alonso et al., 2006) Capture of these physiological events during an UGI would result in a positive study, which may over identify events as abnormal. (Vandenplas et al., 2009)

In a prospective study Aksglaede et al. performed both 24 hour pH study and upper GI contrast study on 21 infants with suspected GORD.(Aksglaede et al., 2003) These infants were studied at a mean age of 3.4 months (range 0.6-8.4) with indications for suspecting GORD being failure to thrive (3/21), recurrent apnoea (9/21) and significant regurgitation (9/21). Although the authors suggest that both pH study and UGI study were performed during one admission, the timing of the studies is not explicitly stated.

Using a threshold for a positive pH study of >5% they found that upper GI contrast studies had a sensitivity of 29% and specificity of 50%. Using the same threshold for a positive pH study did not alter the sensitivity of the results in our study, but reduced the specificity to 40%. Macharia et al. retrospectively compared the results of pH/MII studies and upper GI contrast studies in 66 children and reported a sensitivity of 43% and sensitivity of 24%.(Macharia, 2012)

Determining a cut-off value at which MII/pH studies are considered pathological remains limited by a paucity of normal data. Proposed cut-off values for abnormal pH studies in infants have varied widely. Vandenplas studied 519 healthy infants considered at risk of sudden infant death syndrome, from which the 95$^{th}$ centile for acid index was 10%, and this was proposed as an upper limit of normal.(Vandenplas et al., 1991) In a study of asymptomatic children aged between 2 months and 3 years, Boix-Ochoa derived a lower limit of normal with an acid index

of 5%.(Boix-Ochoa et al., 1980) This criteria was adopted in the study by Aksglaede.(Aksglaede et al., 2003)

There are few data for MII studies in healthy infants, with one study of healthy asymptomatic preterm infants estimating an upper limit of normal as 100 events/day.(Lopez-Alonso et al., 2006) A more recent study used the results of studies from 41 infants investigated for symptoms of GORD, but obtained “normal” values by excluding those with an acid index more than 50% of the upper limit of normal or with a positive symptom index. They reported an upper limit of normal as 79 events/ 24 hours.(Mousa et al., 2014) Using cut off values of acid index of 10% and 79 MII detected reflux events very few of the infants were diagnosed with GORD

This study has strengths and some limitations. It is the first to our knowledge comparing pH/MII study results to upper GI contrast study results. This is a retrospective study evaluating those infants who underwent both investigations during the same admission, but in some cases there was a significant time difference between the two investigations. As GOR is expected to improve with maturity, this may account for some differences between the results, but almost as many UGI studies occurred prior to the pH/MII study. In some cases infants were receiving anti-reflux medication, however those infants were only included in the study if they were on the same medications for both investigations.

The number of infants included in this study is significantly fewer than required by the power calculation, reflected in the large confidence intervals. Accurate estimation of the sensitivity and specificity of UGI contrast studies is therefore not possible.

In this study, there was a poor correlation between the results of UGI study and pH/MII study in infants on the NICU. There was a greater proportion of positive studies by UGI, which may represent an over identification of physiological reflux events as pathological on UGI.

# Chapter 9 : Apnoea and gastro-oesphageal reflux

Apnoea is a common problem on the neonatal intensive care unit, and a frequent indication for initiation of pharmacological therapy. (Clark et al., 2006) Numerous factors are implicated in the pathogenesis, including prematurity, sepsis, anaemia, patent ductus arteriosus and gastro-oesophageal reflux.(Abu-Shaweesh and Martin, 2008). Gastro-oesophageal reflux has been suggested to precipitate apnoea via the laryngeal chemoreflex.(Harding et al., 1976, Menon et al., 1985) The laryngeal chemoreflex (LCR) was first described in 1975 with a study in which water was introduced into the larynx of newborn lambs inducing prolonged apnoea and bradycardia.(Harding et al., 1976) In infants the response is dependent on maturity; prematurely born babies predominantly displaying prolonged apnoea, bradycardia and stridor with few coughs, whereas term babies demonstrate fewer swallows, shorter apnoea and increased cough and arousal. (Boggs and Bartlett, 1982)

Using a fibreoptic laryngoscopy in preterm infants with recurrent apnoea it was noted that secretions frequently pooled in the aryepiglotic folds prior to the onset of apnoea.(Ruggins and Milner, 1991) Gastro-oesophageal reflux has been implicated in the pathogenesis of apnoea(Newell et al., 1989) although evidence for a causative link is limited. As both gastro-oesophageal reflux and apnoea may be more frequent in prematurely born infants, co-occurrence of the two phenomena may not reflect causation.(Poets, 2013) Corvaglia et al. demonstrated a significantly higher frequency of apnoeas following both pH detected and non-acid MII detected reflux events, compared to prior to these events.(Corvaglia et al., 2011b) This contrasts with a number of studies in which a temporal association between apnoea and reflux has not been found.(Barrington et al., 2002, Di Fiore et al., 2010, Harris et al., 2004, Kahn et al., 1992, Moya et al., 2008, Paton et al., 1990, Peter et al., 2002) Studies attempting to determine any association between reflux and apnoea have frequently been limited by methods of detection of both apnoea and of GOR. pH studies only detect acidic reflux events, whereas non-acid reflux events may precipitate apnoea via the laryngeal chemoreflex.(Thach, 2010) Multichannel intraluminal impedance allows detection of reflux events irrespective of pH and may be better equipped to determine temporal association with symptoms.(Peter et al., 2002)

Triggering of the laryngeal chemoreflex by reflux results in reflex closure of the vocal cords, and may be more likely to precipitate obstructive rather than central apnoea and, therefore, a

stronger association between apnoea and reflux may be seen when these apnoeas are assessed independently of central apnoeas.

Several different approaches have been used to assess the temporal association between reflux and apnoea events. In some studies, occurrence of a reflux and apnoea event within a period of time was taken as association, irrespective of the sequence in which they occurred.(Magista et al., 2007) Others have compared the frequency of apnoeas prior to and following a reflux event.(Corvaglia et al., 2011b, Di Fiore et al., 2010) Apnoea frequency during reflux free periods has been used as a further control period,(Corvaglia et al., 2011b) as several studies have suggested that reflux may follow episodes of apnoea.(Omari, 2009, Arad-Cohen et al., 2000) The time window during which events are considered to be associated has varied from 20 seconds(Corvaglia et al., 2011b, Di Fiore et al., 2010) to five minutes. (Barrington et al., 2002, Kahn et al., 1992) Glen et al. developed a novel statistical technique using pH/MII and polysomnography data from four infants selected for having evidence of a temporal association between reflux and apnoea. By altering the relative timing of the pH/MII and polysomnography recordings they generated 10,000 simulated datasets from each patient's recording, and looked at the proportion of those 10,000 that had a higher correlation between apnoea and reflux to quantify the probability that any association was purely by chance.(Glen et al., 2013) They found the strongest temporal association was seen when using a window of two minutes. In the study performed by Ruggins and Milner, however, a delay of up to five minutes was seen between reflux events, pooling of secretions and apparent initiation of the laryngeal chemoreflex and apnoea.(Ruggins and Milner, 1991) In this study I have utilized combined pH and multichannel intraluminal impedance studies, synchronised with polysomnography to evaluate any temporal association between gastro-oesophageal reflux and apnoea.

In this study I have tested several hypotheses. Firstly that both reflux frequency and apnoea frequency will be higher in infants of lower corrected gestational age. Secondly, the frequency of apnoea will be greater in the five-minute period following a reflux event compared to the five minute period preceding it, or a five-minute control period during a reflux free period. Thirdly, any relationship will be most strong when comparing the frequency of obstructive apnoeas rather than to central or mixed apnoeas to reflux events.

## 9.1 Methods

Subjects were recruited from the Neonatal Intensive Care Unit at King's College Hospital NHS Foundation Trust, London between March 2013 and September 2015. The study was approved by the London Riverside Research Ethics Committee. Informed written parental consent was obtained prior to study. Infants of any gestation were eligible for inclusion if they were having recurrent apnoea and the clinician in charge suspected they might be related to GOR. Reasons for suspecting GORD were frequent overt regurgitation, post-prandial apnoea, or discomfort and back-arching.

Infants were only enrolled when they were on full enteral feeds. Infants were excluded if they had known risk factors for apnoea, for example a symptomatic patent ductus arteriosus (the diagnosis confirmed by echocardiography) or suspected sepsis. Infants were not receiving antibiotic treatment and had a normal white cell count and C-reactive protein level in the week preceding the study.

### 9.1.1 Protocol

Studies were performed on the neonatal intensive care in the early morning. The polysomnograph and MII/pH probe were set-up and attached prior to a feed, following which the infant was placed in their usual sleeping position and recordings made for a minimum of two hours.

The MII/pH study and polysomnography were carried out using the equipment described in Chapter 2. The infant's length was measured and oesophageal length was estimated according to Strobel's formula for infants over 40cm (Strobel et al., 1979) and by a nomogram for those under 40cm (Omari et al., 1999). The probe was inserted through a nostril and secured at the required length. A chest radiograph was then obtained to determine if the pH sensor was appropriately positioned between the seventh and ninth thoracic vertebra.(Di Fiore et al., 2009) The position of the probe at the nares was reassessed following completion of the study to ensure the probe had not displaced.

A custom-built synchronisation box provided a synchronisation signal to an auxiliary input into the Alice sleep system, and the multichannel intraluminal impedance system. This synchronization signal was used at the start and end of recording to ensure that the time line for both recordings was equal and synchronised.

### 9.1.2 Analysis

Both pH/MII and polysomnography traces were visually analysed separately. A pH probe reflux event was defined as oesophageal pH less than four for more than five seconds.(Woodley and Mousa, 2006) The total number of pH events / 24 hours was calculated. The duration of the reflux event and the acid clearance time (ACT) (the time from the pH dropping below four to rising above four) were determined. The mean ACT (the total duration with pH <4 divided by the number of acid reflux events) was calculated and the maximum ACT identified. The acid index was the total time spent with the oesophageal pH less than four as a percentage of the total study time. GORD was diagnosed if the acid index was greater than 11.7% (Vandenplas et al., 1991).

MII reflux events were defined as a drop in impedance to less than 50% of the baseline at the most distal channel, which moved retrogradely across at least two channels. These were further classified as either acid (pH <4), weakly acid (4> pH <7) or alkali (pH >7). A pH only event was defined as a drop in oesophageal pH to below 4, without associated changes in impedance sufficient to diagnose an MII reflux event.

Apnoeas were defined as cessation of respiratory airflow for a minimum of five seconds. For each apnoea associated changes in heart rate and oxygen saturations were recorded. The apnoeas were classified as central, obstructive and mixed apnoeas according to the following criteria: central apnoea was defined as a cessation of respiratory airflow and thoraco-abdominal movements for at least five seconds. Obstructive apnoea was defined as a cessation of respiratory airflow with persistence of thoraco-abdominal movements for at least five seconds. Mixed apnoea was defined as a central apnoea and at least one respiratory effort without successful generation of respiratory airflow

An apnoea was considered to be associated with a reflux event if it occurred in a five minute period following the start of a reflux event. The frequency of apnoeas in the five minutes following a reflux event was compared to the frequency of apnoeas in the five minutes preceding the reflux event and to a reflux free control period. The reflux free control period included all periods of the recording occurring more than five minutes from a reflux event.

Data were recorded as the frequency of apnoea per five-minute epoch, either preceding, following or during reflux free periods. Comparisons are made between the frequency of apnoea in the five minute window preceding the start of reflux events and the frequency of apnoea during a five minute window following start of a reflux event, and between the frequency of apnoea during a five minute window following start of a reflux event and during reflux free periods.

### 9.1.3 Statistical analysis

Comparison of apnoea frequency was performed using the Wilcoxon signed rank test. Spearman's correlation coefficients were calculated to determine the strength of the correlations between the frequencies of all apnoeas, central, obstructive and mixed apnoeas, the total reflux events in 24 hours, and corrected gestational age. Partial correlation was subsequently performed to control for corrected gestational age. Analyses were conducted using SPSS Version 22 (SPSS Inc., Chicago, IL, USA).

### 9.1.4 Sample size

In order to detect a within patient difference in the number of apnoeas before and after GOR of 0.3 standard deviations with power 80% and significance level 5%, a similar size of difference to that reported by Corvaglia,(Corvaglia et al., 2011b) a sample size of 60 infants was required.

## 9.2 Results

Forty infants were recruited to the study born at a median gestation of 29 (range 24 to 42) weeks studied at a median postnatal age of 53 (range 2 to 212) days, with a median corrected gestational age of 37 (range 30-54) weeks. Each infant underwent a median study duration of three hours (range 2 to 5 hours). In three infants the pH/MII probe was found to have dislodged at completion of the recording. The data from those recordings were not included in analysis, but each infant underwent the protocol successfully on a subsequent day.

A total of 123 hours of recordings were analysed which included 900 central apnoeas, 580 obstructive apnoeas and 452 mixed apnoeas. One hundred and fifty-three pH only events, 344 non-acid MII events and 37 acid MII events were recorded. Of the 1932 apnoeas, seven hundred and forty-five occurred in the five minutes preceding a reflux event, 754 in a five minute period following a reflux event and 439 during a reflux free period. Both apnoea index and total reflux events were inversely correlated with corrected gestational age ($\rho$=-0.47, p = 0.002 (Figure 21) and $\rho$=-0.41, p = 0.010 (Figure 22) respectively).

Apnoea index and total reflux events significantly correlate ($\rho$=0.34, p = 0.034). (Figure 23)

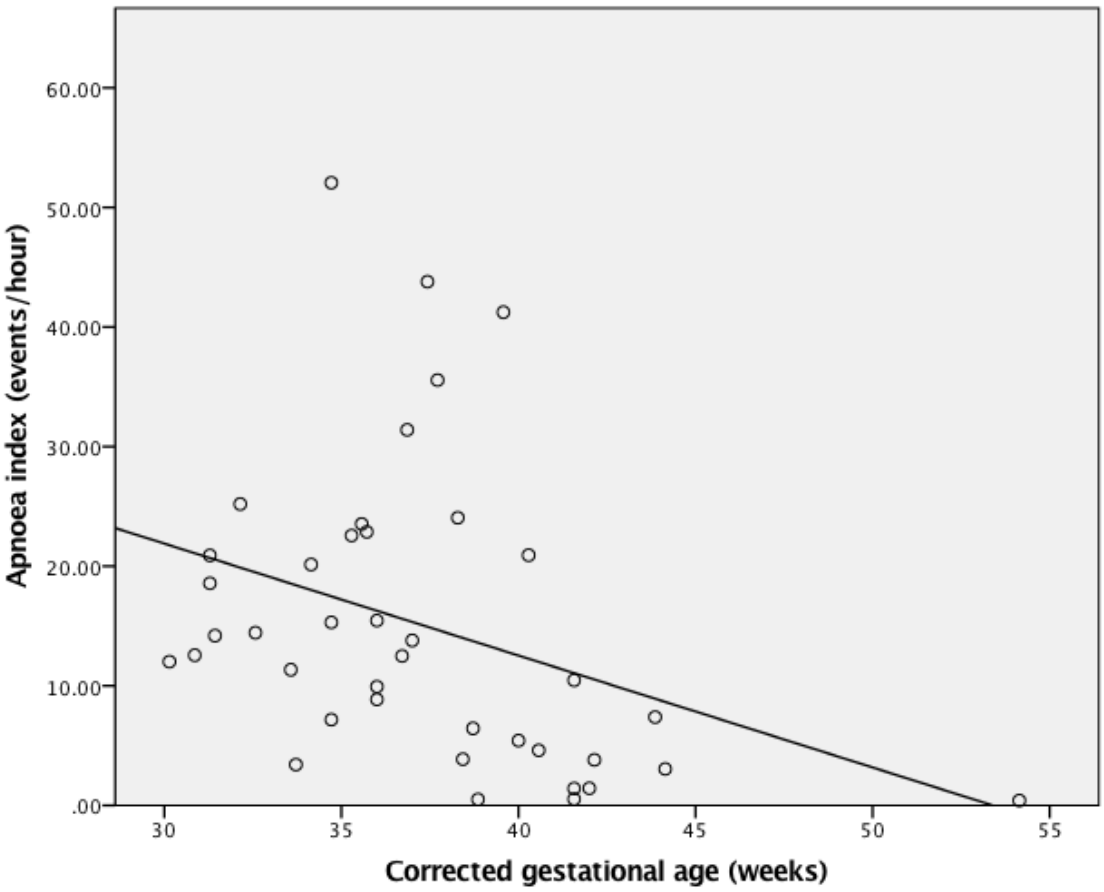


Figure 21: Scatter plot of apnoea index against corrected gestational age showing a significant negative correlation

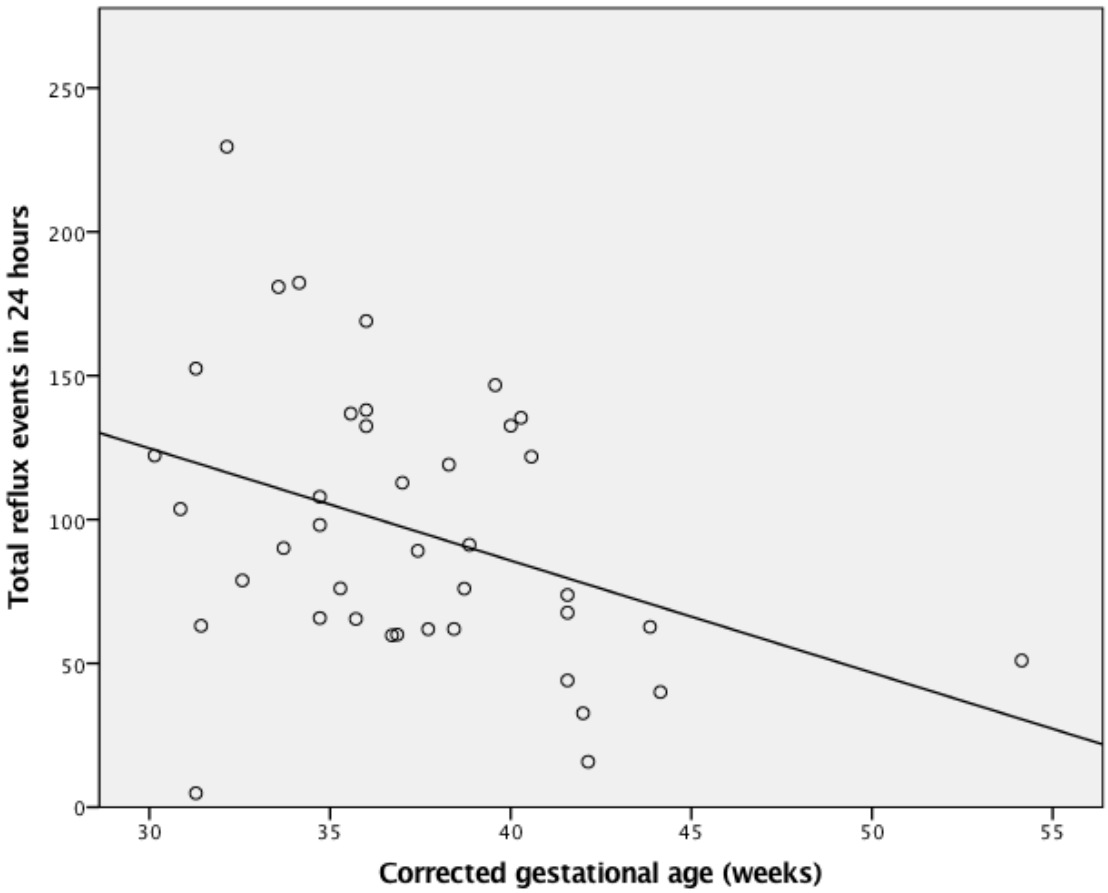


Figure 22: Scatter plot of reflux events/24 hours plotted against corrected gestational age showing a significant negative correlation

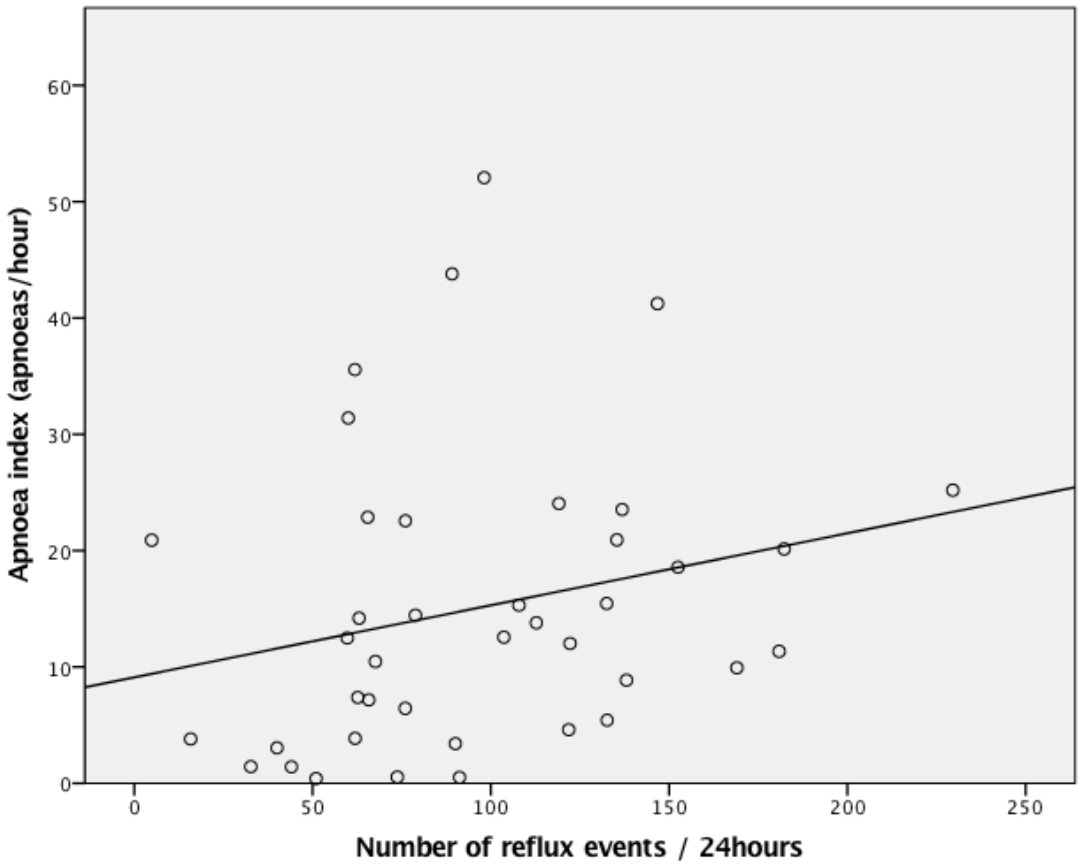


Figure 23: Scatter plot of Apnoea index against total number of reflux events in 24 hours showing a signifcant positive correlation

Central apnoea index correlated significantly with total number of reflux events (ρ=0.34, p = 0.030). Obstructive and mixed apnoea indices did not correlate with total number of reflux events.

When controlling for corrected gestational age using partial correlation, there was no longer a significant correlation between total reflux events and either total apnoea index or central apnoea index.

Overall, there were no significant differences between the frequency of apnoeas pre and post reflux, nor between the frequency of apnoeas post-reflux and during reflux free periods. (Table 31) The frequency of obstructive apnoeas was significantly higher before reflux events compared to a period following reflux events (p=0.03).

When only pH detected acid events were analysed there was no difference between the frequency of apnoeas of any type before and after acid reflux event, or during a reflux free period.(Table 32)

No significant differences between apnoea frequency were seen before or after acid MII detected reflux events or after acid MII events and a reflux free control period. (Table 33)

The frequency of obstructive apnoeas was higher before MII detected weakly acid reflux events than following MII detected weakly acid reflux events (p=0.04). (Table 34)

| | Pre reflux | Post reflux | Reflux free | p-value (pre vs post reflux) | p-value (post reflux vs reflux free period) |
|---|---|---|---|---|---|
| All apnoeas | 0.94 (0-6) | 0.97 (0-4.58) | 0.87 (0-4.04) | 0.38 | 0.77 |
| Central apnoea | 0.31 (0-3.44) | 0.4 (0-2.50) | 0.35 (0-2.4) | 0.75 | 0.90 |
| Mixed apnoea | 0.20 (0 - 2) | 0.20 (0-1) | 0.17 (0-1.19) | 0.41 | 0.64 |
| Obstructive apnoea | 0.26 (0-2.5) | 0.15 (0-2.5) | 0.17 (0-1.60) | 0.03 | 0.98 |

Table 31: Frequency of apnoeas in 5 minute periods preceding reflux, following reflux, and the mean frequency during reflux free periods. Data are expressed as median (range)

| | Pre pH reflux | Post pH reflux | Reflux free | p-value (pre vs post reflux) | p-value (post reflux vs reflux free period) |
|---|---|---|---|---|---|
| Central apnoea | 0.33 (0-3.25) | 0.44 (0-3) | 0.35 (0-2.4) | 0.38 | 0.06 |
| Mixed apnoea | 0.21 (0-2) | 0 (0-1.56) | 0.17 (0-1.19) | 0.25 | 0.71 |
| Obstructive apnoea | 0.21 (0-2) | 0 (0-5) | 0.17 (0-1.60) | 0.42 | 0.20 |
| All apnoeas | 1 (0-6) | 0.95 (0-7) | 0.87 (0-4.04) | 0.42 | 0.43 |

Table 32: Frequency of apnoeas in 5 minute periods preceding pH reflux, following pH reflux, and the mean frequency during reflux free periods. Data are expressed as median (range)

| | Pre MII acid reflux | Post MII acid reflux | Reflux free | p-value (pre vs post reflux) | p-value (post reflux vs reflux free period) |
|---|---|---|---|---|---|
| Central apnoea | 0.25 (0-4) | 0 (0-4.5) | 0.35 (0-2.4) | 0.86 | 0.24 |
| Mixed apnoea | 0 (0-1) | 0 (0-1) | 0.17 (0-1.19) | 0.14 | 0.81 |
| Obstructive apnoea | 0 (0-1.5) | 0 (0-1) | 0.17 (0-1.60) | 0.61 | 0.87 |
| All apnoeas | 1 (0-6) | 0.75 (0-5) | 0.87 (0-4.04) | 0.92 | 0.80 |

Table 33: Frequency of apnoeas in 5 minute periods preceding MII acid reflux, following MII acid reflux, and the mean frequency during reflux free periods. Data are expressed as median (range)

| | Pre MII weakly acid reflux | Post MII weakly acid reflux | Reflux free | p-value (pre vs post reflux) | p-value (post reflux vs reflux free period) |
|---|---|---|---|---|---|
| Central apnoea | 0.21 (0-3.4) | 0.27 (0-2.4) | 0.35 (0-2.4) | 0.52 | 0.29 |
| Mixed apnoea | 0.1 (0-1) | 0.23 (0-1.25) | 0.17 (0-1.19) | 0.05 | 0.56 |
| Obstructive apnoea | 0.46 (0-2.63) | 0.14 (0-2.27) | 0.17 (0-1.60) | 0.04 | 0.51 |
| All apnoeas | 1 (0-5.18) | 0.93 (0-4.45) | 0.87 (0-4.04) | 0.29 | 0.82 |

Table 34: Frequency of apnoeas in 5 minute periods preceding MII acid reflux, following MII acid reflux, and the mean frequency during reflux free periods. Data are expressed as median (range)

## 9.3 Discussion

This study has demonstrated a positive correlation between corrected gestational age and both the frequency of apnoea and reflux events. The absence of a temporal association between reflux episodes and subsequent apnoea suggests that they are not causally related. No evidence was found to support the hypothesis that gastro-oesophageal reflux would result in obstructive and mixed apnoeas. Despite studying a population in which the clinician had a high index of suspicion of gastro-oesophageal reflux related respiratory disturbance, no temporal association suggestive of causation could be demonstrated.

The results of this study contrast with several studies in which an association between apnoea and reflux has been detected.

Menon et al. studied nine preterm and one term infant with a history of regurgitation and apnoea using pH study and polysomnography.(Menon et al., 1985) They found that apnoea was more common during episodes of regurgitation compared to control periods during which no regurgitation was evident. They noted that while some apnoeas were related to regurgitation the majority of apnoeas were not. As regurgitation was noted by report rather than a synchronised recording, it is difficult to determine if the apnoeas occurred prior to or after the regurgital events. Several studies have suggested that apnoea may be associated with reduced lower oesophageal sphincter tone,(Arad-Cohen et al., 2000, Omari, 2009) increasing the likelihood of subsequent reflux and regurgitation events which may explain the findings in this earlier study. This is supported by our finding that obstructive apnoeas were more frequent before weakly acid MII events than after.

Corvaglia's results are in contrast to the present findings.(Corvaglia et al., 2011b) There are differences between the studies, in that Corvaglia's study population included all infants with apnoea of prematurity. Secondly, the window of association was significantly smaller than used in this study. Both of these differences would be expected to increase the likelihood of an association being detected in our population.

Magista et al. studied 6 preterm infants, and found an increased frequency of apnoea during periods of gastro-oesophageal reflux, particularly with weakly acid reflux events and reflux events of longer duration.(Magista et al., 2007) Association was defined by the onset of reflux and apnoea within 20 seconds of each other. Again, there was no significant difference in the frequency of apnoea in the epochs preceding and following a reflux event.

A number of studies have failed to detect a temporal association between reflux events and apnoea. Paton et al. used pH studies and polysomnography to study 24 infants aged between 6 and 10 weeks with respiratory abnormalities comprising frequent desaturation, bradycardia or apnoea. No association was demonstrated between these respiratory events and pH detected reflux.(Paton et al., 1990)

Kahn et al. studied a cohort of 50 infants aged between four and twenty-six weeks referred for apparent life-threatening events, and 50 control infants that underwent polysomnography and pH study. The frequency of apnoea in the five minute window following a reflux event was no greater than that in the period preceding it.(Kahn et al., 1992) Barrington et al. studied 45 prematurely born infants at term, using pH study and polysomnography.(Barrington et al., 2002) They found no difference in the frequency of apnoea following a reflux event, compared to preceding an event. Similarly to our study they used a 5 minute window in which to detect an association. In contrast to our study, they found no correlation between apnoea frequency and reflux frequency, however as the infants were studied at or around term, the covariate effect of immaturity would not be apparent.

The present results are in keeping with those of Wenzl et al. (Wenzl et al., 2001) who studied 22 infants with a mean age of two months, with suspected GORD using pH/MII and polysomnography, and found that a small proportion of apnoea occurred in association with reflux. An association was defined as apnoea occurring within 30 seconds of a reflux episode, either before or after. They found there was no difference in the frequency of apnoea following reflux compared to preceding reflux. In their study they similarly found a correlation between the number of apnoeic events and number of reflux events.

Peter et al. using MII/pH studied nineteen infants with a median corrected gestational age of 33 weeks, with a diagnosis of apnoea of prematurity, and found no temporal association, studying each infant for six hours, and using an “association” window of 20 seconds.(Peter et al., 2002) Di Fiore studied 71 prematurely born infants at term using pH/MII and polysomnography,(Di Fiore et al., 2010) and found that while only 3% of cardio-respiratory events (apnoea, desaturation or bradycardia) followed reflux events, 9% of reflux events were preceded by a cardiorespiratory event. Again they concluded that reflux was not a significant contributor to the development of apnoea. Funderburk et al. studied 40 preterm and 18 term infants using pH/MII suspected to have GORD.(Funderburk et al., 2016) Symptom correlation was carried out by means of a symptom diary completed by nursing staff and parents. There was no evidence of correlation between reflux events and apnoea. Nunez et al. studied seven infants born preterm who had persistent cardio-respiratory disturbances post term. The infants were studied between 39 and 48 weeks post-menstrual age using a combination of MII/pH and polysomnography, and classified the type of apnoea. Overall, there was no significant increase in the odds of obstructive apnoea occurring following a reflux event compared to in the absence of an event.(Nunez et al., 2011)

Several studies have utilized a variety of approaches including symptom index, symptom sensitivity index and symptom association probability to determine association on an individual basis. These techniques have several limitations not least the likelihood of type 1 errors when studying large groups, as in the absence of symptom association there will be a 5% probability of falsely rejecting the null hypothesis.(Glen et al., 2013) Mousa et al. studied 25 patients aged between one and nineteen months with polysomnography and MII/pH and found no temporal association. Mousa et al. used a 5 minute window of association as in our study. They found no association across the patient group, but positive symptom association using chi-squared tests within four of the twenty five individual patients.(Mousa et al., 2005) In the study by Nunez et al. in which no increase in the odds of an apnoea following a reflux event compared to in the absence of reflux, three of the infants had a positive symptom association probability.(Nunez et al., 2011)

This study has strengths and some limitations. Respiratory events were comprehensively assessed using polysomnography rather than relying on manual recording of events. Furthermore, use of a synchronization signal between the polysomnograph and pH/MII recorder ensures accuracy in the correlation of respiratory and reflux events. The use of pH/MII to detect reflux ensured comprehensive detection of both acid and non-acid reflux events. By using polysomnography to monitor for respiratory events I was able to classify the type of apnoea and assess for temporal association between reflux events The study population was heterogeneous with regards to gestational age at birth, and corrected gestational age at study, but were selected for study based on a high clinical suspicion of respiratory disturbance caused by GORD.

This study has demonstrated that both reflux indices and apnoea indices correlate with prematurity, but there was no evidence that apnoea was precipitated by reflux events. However, there was some evidence to suggest that obstructive apnoeas may precipitate weakly acid reflux events.

# Chapter 10 : Summary

The key findings in this thesis are:

## 10.1 The effect of maternal smoking and substance misuse and infant sleeping position on the ventilatory response to hypercarbia in the newborn period

Prone sleeping in infants of mothers who smoked or misused substances in pregnancy alters resting respiratory characteristics compared to when sleeping supine. This is not seen in infants of mothers who have not smoked or misused substances, which may account for the increased risk of prone sleeping in these at risk groups. Infants of substance misusing mothers have a higher resting minute volume, and lower end-tidal carbon dioxide level than those of smoking and control mothers. The ventilatory response to hypercarbia was not significantly altered either by sleeping position or maternal smoking or substance misuse in pregnancy.

## 10.2 The effects of sleeping position and maternal smoking and substance misuse on the ventilatory response to hypoxia in the newborn period

When exposed to hypoxia the rate of decline in minute ventilation is significantly greater in infants of substance misusing mothers compared to infants of smoking mothers or controls while sleeping prone but not supine. There were no other significant differences in the response to hypoxia either between the prone and supine sleeping positions, or between infants of substance misusing mothers, infants of smoking mothers and controls. These studies have demonstrated that both sleeping position and maternal substance misuse significantly alter baseline respiratory characteristics. However they did not support the hypothesis that an impaired ventilatory response to hypoxia explains the increased risk of SIDS in the presence of these factors.

## 10.3 The effect of caffeine on the ventilatory response to hypercarbia in preterm infants

In this study I demonstrated that prematurity is associated with a weaker ventilatory response to hypercarbia, and this response increases with increasing maturity. Furthermore, I demonstrated that the ventilatory response to hypercarbia is stronger when receiving caffeine therapy. Infants studied in the immediate newborn period had few apnoeas, and the number of apnoea at this time point did not predict the frequency of apnoeas subsequently. The ventilatory response to hypercarbia was significantly lower in those infants that went on to require caffeine therapy for apnoea of prematurity, compared to those that did not. This study provides strong support for the hypothesis that a weaker ventilatory response to hypercarbia is contributive to apnoea of prematurity, and that increasing the sensitivity to hypercarbia may be a mechanism by which caffeine imparts benefit.

## 10.4 Investigation and management of gastro-oesophageal reflux in neonatal intensive care units in the United Kingdom

I have demonstrated there is no consistency regarding investigation or medication use in infants with suspected GORD in neonatal units in the UK. This likely reflects a limited evidence base and highlights the need for appropriate studies to inform best practice.

## 10.5 Detection of gastro-oesophageal reflux on the neonatal unit

There is poor correlation between a clinical diagnosis of GORD, and results of pH/MII study. Multichannel intraluminal impedance increased the detection of GOR compared to pH study alone. Baseline intraluminal impedance measurements, a marker of mucosal integrity, correlates negatively with acid reflux indices, but not non-acid reflux indices, suggesting the former are of greater significance in infants in causing oesophageal damage.

## 10.6 Comparison of the diagnosis of gastro-oesophageal reflux in neonatal units by combined pH/multichannel intraluminal impedance studies and upper gastrointestinal contrast studies

There is poor correlation between the results of Upper GI contrast studies and pH/MII study. Gastro-oesophageal reflux was frequently diagnosed by Upper GI contrast study in infants with normal pH and MII studies, resulting in poor specificity and positive predictive value compared to pH/MII.

## 10.7 Apnoea and gastro-oesphageal reflux

While apnoea frequency and GOR frequency correlate, there is no evidence that GOR precipitates apnoea. This suggests that apnoea is not a symptom of GOR in infants on the NICU, and GORD treatment should not be initiated on this indication.

## 10.8 Research potential for the future

In this thesis infants exposed to risk factors for SIDS were studied in the newborn period prior to discharge from the postnatal ward. It would be invaluable to examine the ventilatory responses in these infants at the high risk age for SIDS to examine how the effects of maternal smoking and substance misuse, and prone sleeping change during this time period.

There is evidence of brainstem changes in infants who die of SIDS compared to controls who died of other causes.(Kinney et al., 2009) Non-invasive measures of brain stem function such as auditory evoked brainstem responses may provide an interesting research tool in the evaluation of these infants to determine the effect of antenatal risk factors. This technique has been widely used as part of the newborn hearing screen for over a decade.(Kennedy, 2000) Future research may use this technique prospectively to explore the effect of maternal smoking and substance misuse on brainstem conduction. Furthermore, after 15 years of widespread use as a screening tool there may be sufficient data to retrospectively compare brainstem conduction in infants who later succumbed to SIDS compared to controls.

Effective prediction of those infants at risk of neonatal apnoea may allow targeted use of caffeine therapy. A prospective study using carbon dioxide sensitivity as a predictor of neonatal apnoea would evaluate the findings of this study. If this remains a strong predictor then a more clinically practical method of measuring the ventilatory response to hypercarbia must be developed to make this a practicable tool in the NICU.

The results of the studies examining the investigation and management of GOR continue to raise questions regarding the clinical relevance of investigations, and whether GORD is a useful focus of treatment. The absence of a strong association between reflux events and subsequent apnoea strengthens the case against treatment of respiratory disturbance with anti-reflux medication. Future research could evaluate long-term outcomes in those infants with a high clinical suspicion in a placebo-controlled study of anti-reflux medication.

# Appendix A. Investigation and management of gastro-oesophageal reflux disease: Survey

**Survey monkey: The diagnosis and treatment of gastro-oesophageal reflux disease on the NICU**

1. Which of the following modalities do you use for the detection and diagnosis of gastro-oesophageal reflux disease. Please rank those that you use on your NICU in terms of usefulness (1 = most useful)

- pH study
- Combined Multichannel intraluminal impedance/pH study
- Upper GI contrast study
- Ultrasounds
- Nuclear scintigraphy
- Trial of therapy

2. If you use pH studies, what acid index would you consider requires treatment?

- >5%
- >7%
- >10%
- pH Studies not used
- Other

3. If you use MII/pH how many reflux events in 24 hours would you consider abnormal? (*these levels have been reported in the literature)

- >50*
- >73*
- >100*
- MII/pH studies not used
- Other (please specify)

4. How often are infants commenced on anti-reflux medication without investigation?

- Never
- Occasionally
- Often
- Always

5. What is your first line treatment for gastro-oesophageal reflux disease? (you may select more than one option)

- Thickening agents (carob gum etc.)
- Domperidone
- Metoclopramide
- Cisapride
- Erythromycin
- Antacids (e.g. Aluminium or magnesium hydroxide)
- Gaviscon
- Sucralfate
- H2 Receptor blocker (e.g. Ranitidine)
- Proton pump inhibitors (e.g. Omeprazole)

6. What would be your criteria for stopping treatment?

- Resolution of symptoms
- Discharge
- First outpatient review
- Other (please specify)

7. Which neonatal unit do you work for?

# Appendix B. Ethical Approval

The following ethical approvals were obtained prior to undertaking the studies performed in this thesis.

Study title: The effect of maternal smoking, substance misuse and infant sleeping position on ventilatory control and sleep pattern in the newborn

REC reference: 13/LO/0054

IRAS project ID: 115493

Study title: The effect of prematurity, and caffeine therapy on central chemoreceptor sensitivity and respiratory pattern in the newborn.

REC reference: 13/LO/0053

IRAS project ID: 118721

Study Title: Exploring the role of acid and non-acid reflux in apnoea

REC reference: 13/LO/1099

IRAS project ID: 122897

# Appendix C. Patient information sheets

Information about the research (Control group)

**The effects of smoking and substance misuse in pregnancy and sleeping position on the breathing and sleep pattern of babies**

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Part 1 tells you the purpose of this study and what will happen to your baby if you take part. Part 2 gives you more detailed information about the conduct of the study. Please ask us if there is anything that is not clear.

**Part 1:**

**Why are we doing this study?**

Babies born to mothers who have used substances or smoked during pregnancy are at a higher risk of Sudden Infant Death Syndrome (also known as 'cot death'), particularly if they sleep on their front. We know that babies sleeping on their front sleep deeper and wake up during sleep less frequently than when they sleep on their back.

As a consequence we would like to study how maternal substance use, maternal smoking and sleeping position affect babies' breathing and sleep pattern. This may help us understand why some babies sadly die of Sudden Infant Death Syndrome.

**How will we do it?**

As you have not used substances or smoked we wish to study your baby as a control (comparator) infant and we can then compare the results obtained from your baby with those born to mothers who used substances and/or smoked during pregnancy.

We would ask you to provide us with a 10 ml sample of your urine. This would allow us to measure the cotinine level, which is a by-product of smoking, in the urine. We will not take any blood from your baby but we would like to have a urine sample from your baby at birth and two months of age. This will help us to see whether there is any cotinine present in your baby at birth and if it remains six weeks later.

In the first week after birth, we wish to measure your baby's breathing. This will involve placing a soft mask over the face for a few minutes while they sleep. This will measure your baby's breathing and allow us to make small changes to the amount of oxygen and carbon dioxide (the waste gas) in the air they breathe in. We will slightly increase the amount of carbon dioxide the baby breathes in and very slightly reduce the amount of oxygen your baby breathes to the same level as if they were on an aeroplane flight. This is a standard test called "Fit to Fly" and is routinely used to see if prematurely born babies can go on an aeroplane.

We will also closely monitor how your baby sleeps, and how often they wake up.

At two months of age we wish to repeat the breathing tests described above. This will take between one and two hours while your baby sleeps.

**Are there any risks in taking part in the study?**

There are no known risks to your baby taking part in this study. These measurements have been carried out in other babies and no adverse effects have been seen. Your baby will be monitored throughout the test and if the monitoring shows the oxygen levels have dropped the test will be stopped immediately.

**Are there any benefits in taking part in the study?**

The information we get from this study will help improve our understanding of breathing problems in babies. If your baby's oxygen saturation level does fall during the test, we will advise you not to take your baby on an aeroplane for a further three months, and with your permission advise your General Practitioner that a further test should be undertaken to determine when your baby is fit to fly.

We will reimburse travel costs for the follow-up visit.

**Part 2:**
**What will happen if I wish to withdraw from the study?**

You may withdraw your child at any time without their medical care or legal rights being affected. If this was to happen, we will give you the choice as to whether we may use any information gathered up to that point.

**How will the information be shared?**
Results from the research study will be presented in the scientific meetings and published in the scientific journals. All data will be reported anonymously; the names and identifiable information about the individuals will not be disclosed.

We would like to inform your GP of your participation in this study.

**Giving consent**
It is important for you to understand that this study is voluntary and for research purposes. You may choose not to allow your child to participate in this study and. If you require any further information please ask. There are no harmful side effects associated with this study. However, indemnity is provided by King's College London.

If you decide to allow your child to take part in the study you will be given a copy of this information sheet and consent form to keep. All information that is collected during the course of the research will be kept strictly confidential and if the results are published then anonymity will be maintained.

This study was reviewed and approved by Bromley research ethics committee 13/LO/0054.
Thank you very much for considering your child for enrolment into this project. If you are happy to participate please sign the attached consent form.

If you have any further questions, or would like to meet with a member of the research team to discuss this further, please do not hesitate to contact us on the telephone number below.

Dr Thomas Rossor 0203 299 9000 ext 8495
Professor Anne Greenough 0203 299 3037

Information about the research (Substance or smoking group)

**The effects of smoking and substance misuse in pregnancy and sleeping position on the breathing and sleep pattern of babies**

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Part 1 tells you the purpose of this study and what will happen to your baby if you take part. Part 2 gives you more detailed information about the conduct of the study. Please ask us if there is anything that is not clear.

**Part 1:**

**Why are we doing this study?**

Babies born to mothers who have used substances or smoked during pregnancy are at a higher risk of Sudden Infant Death Syndrome (also known as 'cot death'), particularly if they sleep on their front. We know that babies sleeping on their front sleep deeper and wake up during sleep less frequently than when they sleep on their back.

As a consequence we would like to study how maternal substance use, maternal smoking and sleeping position affect babies' breathing and sleep pattern. This may help us understand why some babies sadly die of Sudden Infant Death Syndrome.

**How will we do it?**

We would be very grateful if you would complete a questionnaire which asks about your smoking and substance use habits. We would ask you to provide us with a 10 ml sample of your urine. This would allow us to measure any drug levels, and the cotinine level, which is a by-product of smoking, in the urine. We do not need to take any blood from your baby but we would like to get a urine sample from your baby at birth and two months of age. This will help us to see whether any drugs were present in your baby at birth and if any remains six weeks later.

In the first week after birth, we wish to measure your baby's breathing. This will involve placing a soft mask over the face for a few minutes while they sleep. This will measure your baby's breathing and allow us to make small changes to the amount of oxygen and carbon dioxide (the waste gas) in the air they breathe in. We will slightly increase the amount of carbon dioxide the baby breathes in and very slightly reduce the amount of oxygen your baby breathes to the same level as if they were on an aeroplane flight. This is a standard test called "Fit to Fly" and is routinely used to see if prematurely born babies can go on an aeroplane.

We will also closely monitor how your baby sleeps, and how often they wake up.

At two months of age we wish to repeat the breathing tests described above. This will take between one and two hours while your baby sleeps.

**Are there any risks in taking part in the study?**

There are no known risks to your baby taking part in this study. These measurements have been carried out in other babies and no adverse effects have been seen. Your baby will be monitored throughout the test and if the monitoring shows the oxygen levels have dropped the test will be stopped immediately.

**Are there any benefits in taking part in the study?**

The information we get from this study will help improve our understanding of breathing problems in babies. If your baby's oxygen saturation level does fall during the test, we will advise you not to take your baby on an aeroplane for a further three months, and with your permission advise your General Practitioner that a further test should be undertaken to determine when your baby is fit to fly.

We will reimburse travel costs for the follow-up visit.

**Part 2:**

**What will happen if I wish to withdraw from the study?**

You may withdraw your child at any time without their medical care or legal rights being affected. If this was to happen, we will give you the choice as to whether we may use any information gathered up to that point.

**How will the information be shared?**

Results from the research study will be presented in the scientific meetings and published in the scientific journals. All data will be reported anonymously; the names and identifiable information about the individuals will not be disclosed.

We would like to inform your GP of your participation in this study.

**Giving consent**
It is important for you to understand that this study is voluntary and for research purposes. You may choose not to allow your child to participate in this study and. If you require any further information please ask. There are no harmful side effects associated with this study. However, indemnity is provided by King's College London.

If you decide to allow your child to take part in the study you will be given a copy of this information sheet and consent form to keep. All information that is collected during the course of the research will be kept strictly confidential and if the results are published then anonymity will be maintained.

This study was reviewed and approved by Bromley research ethics committee 13/LO/0054.
Thank you very much for considering your child for enrolment into this project. If you are happy to participate please sign the attached consent form.

If you have any further questions, or would like to meet with a member of the research team to discuss this further, please do not hesitate to contact us on the telephone number below.

Dr Thomas Rossor 0203 299 9000 ext 8495
Professor Anne Greenough 0203 299 3037

Information about the research:

**The effect of prematurity on breathing patterns in babies**

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Part 1 tells you the purpose of this study and what will happen to your baby if you take part. Part 2 gives you more detailed information about the conduct of the study. Please ask us if there is anything that is not clear.

**Part 1:**

**Why are we doing this study?**
Babies born prematurely have different breathing patterns to those born at term. They may respond less to breathing carbon dioxide, the waste gas we breathe out, and have more pauses in their breathing. We would like to study your baby to assess his/her breathing and sleep pattern.

**How will we do it?**

Soon after your baby is born, and if they are not requiring any help with their breathing, we would like to measure your babies breathing pattern, and response to breathing a slightly increased amount of carbon dioxide.
We will measure your baby's breathing by placing a soft mask over their face for a few minutes while they sleep. This will measure the baby's response to small changes in the amount of carbon dioxide in the air they breathe in. We will also closely monitor how your baby sleeps, and how often they wake up. None of this is painful or distressing for your baby, but it does involve attaching several sticky sensors to your baby's head and body to measure sleep. Your baby will continue to wear the face mask so we can monitor their breathing, this may last up to two hours.
The breathing pattern of your baby will change over the next weeks, and we would like to repeat the studies every week while your baby is an inpatient on the neonatal unit. Once your baby has left the newborn unit they will not be followed up by the research team, but will be seen by the clinical team as part of routine follow up.

**Are there any risks in taking part in the study?**
There are no known risks to participants in this study. The measurements are safe and won't upset your baby and no adverse effects have been reported in the many studies that have previously been undertaken.

**Are there any benefits in taking part in the study?**

The information from this study will help improve our understanding of breathing problems in preterm babies and if we find an abnormality of breathing pattern in your baby we will recommend to your baby's consultant your baby continues on caffeine until the results of the studies show no abnormalities.

**Part 2:**

**What will happen if I wish to withdraw from the study?**

You may withdraw your child at any time without their medical care or legal rights being affected. If this was to happen, we will give you the choice as to whether we may use any information gathered up to that point.
We would like to inform your GP of your participation in this study but would only do this with your permission.

**How will the information be shared?**
Results from the research study will be presented in the scientific meetings and published in the scientific journals, all data will be reported anonymously the names and identifiable information about the individuals will not be disclosed.

**Giving consent**
It is important for you to understand that this study is voluntary and for research purposes. You may choose not to allow your child to participate in this study and you may withdraw your child at any time without their medical care or legal rights being affected. If you require any further information please ask. There are no harmful side effects associated with this study. No indemnity is available, although, normal NHS procedures apply in the unlikely event of any adverse outcome of the study.
If you decide to allow your child to take part in the study you will be given a copy of the information sheet and consent form to keep. All information that is collected during the course of the research will be kept strictly confidential and if the results are published then anonymity will be maintained. This study was reviewed and approved by the Bromley Research Ethics Committee: Reference 13/LO/0053.
Thank you very much for considering your child for enrolment into this project. If you are happy to participate please sign below. If you have any further questions, or would like to meet with a member of the research team to discuss this further, please do not hesitate to contact us on the telephone number below.

Dr Thomas Rossor 0203 299 8495
Professor Anne Greenough 0203 299 3037

Information about the research:

**Gastro-oesophageal reflux and apnoea study**

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Part 1 tells you the purpose of this study and what will happen to your baby if you take part. Part 2 gives you more detailed information about the conduct of the study. Please ask us if there is anything that is not clear.

**Part 1:**

**Why are we doing this study?**

Gastro-oesophageal reflux (regurgitation of stomach acid or milk back up the oesophagus (food pipe)) is very common in babies. It is possible that such reflux may result in pauses in breathing (apnoea) or drops in oxygen levels (desaturations). We would like to find out if the episodes of reflux are related to apnoeas and/or desaturations.

**How will we do it?**

If your baby is having episodes of apnoea or desaturation without any obvious cause, we would like to study your baby to see if the episodes may be caused by reflux.

We will assess whether your baby has reflux using a probe which is very similar to a naso-gastric feeding tube. This is passed through the nose to sit at the bottom of the oesophagus, secured in the same manner as a feeding tube, and connected to a recording device. This will monitor reflux.

While we monitor for reflux episodes we will also record your babies breathing pattern, heart rate and oxygen saturations. We will do this by attaching sensors under your babies nose, on their chest and foot. These will be recorded on a computer, as well as a video of your baby's activity will be made. Your baby will be recorded for at least two hours.

After your baby has been studied, we will analyse the recording and be able to inform the clinical team of any reflux that is occurring.

We would like to repeat the study on one more occasion after a week to see if there are any changes with age.

Once your baby has left the newborn unit, they will not be followed up by the research team, but will be seen again by the clinical team as they think necessary.

**Are there any risks in taking part in the study?**

There are no known risks to participants in this study. The measurements are safe. Passing the tube through the nose is briefly uncomfortable, but no more so than passing feeding tubes .

**Are there any benefits in taking part in the study?**

The study uses a new technique to monitor reflux which will provide more information than other techniques that are available, this may allow improved treatment of your baby.

**Part 2:**

**What will happen if I wish to withdraw from the study?**

You may withdraw your child at any time without their medical care or legal rights being affected. If this was to happen, we will give you the choice as to whether we may use any information gathered up to that point.

**How will the information be shared?**

Results from the research study will be presented in the scientific meetings and published in the scientific journals, all data will be reported anonymously the names and identifiable information about the individuals will not be disclosed.

We would like to inform your GP of your participation in this study.

**Giving consent**

It is important for you to understand that this study is voluntary and for research purposes. You may choose not to allow your child to participate in this study and. If you require any further information please ask. There are no harmful side effects associated with this study. However, indemnity is provided by King's College London.

If you decide to allow your child to take part in the study you will be given a copy of this information sheet and consent form to keep. All information that is collected during the course of the research will be kept strictly confidential and if the results are published then anonymity will be maintained.

This study was reviewed and approved by London Riverside Research ethics committee ref.13/LO/1099.
Thank you very much for considering your child for enrolment into this project. If you are happy to participate please sign the attached consent form.

If you have any further questions, or would like to meet with a member of the research team to discuss this further, please do not hesitate to contact us on the telephone number below.

Dr Thomas Rossor 0203 299 9000 ext 8495
Professor Anne Greenough 0203 299 3037

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